Prevalence and clinical relevance of helminth and tuberculosis co-infection in children under five years of age in Tanzania

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Khadija S. Ibrahim

von Tanzania

Basel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

Prof. Marcel Tanner, PhD, Prof. Lukas Fenner, MD and Prof. Christopher Hatz, PhD

Basel, October 17, 2017

Prof. Dr. M. Spiess
The Dean of Faculty of Science

Dedicated to my dear family

Faculty representative
Prof. Marcel Tanner, PhD
Swiss Tropical and Public Health Institute
Basel
Switzerland

Supervisor
Prof. Lukas Fenner, MD
Institute of Social and Preventive Medicine,
University of Bern
Bern
Switzerland

Co-referee
Prof. Christoph Hatz, MD
Swiss Tropical and Public Health Institute
Basel
Switzerland

Financial support

This work was financially supported by the Swiss Government Excellence Scholarship (ESKAS), the Rudolf Geigy-Stiftung, Swiss Tropical and Public Health Institute and the Ifakara Health Institute. I am very grateful to all the donors for their generous funding.

Table of Contents

Table of Contents

Table of Contents

List of figures

List of tables

List of supplementary tables

List of supplementary tables

List of publications

Manuscript I

***Schistosoma,* other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania**

Manuscript II

Immunologic-based Diagnosis of Latent Tuberculosis among Children Less Than 5 Years of Age Exposed and Unexposed to Tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening

Manuscript III

Impact of Infections on Growth Development, Micronutrient Status and Cognitive function in Pre-School Aged Children in Tanzania

Additional work performed during the PhD not directly related to this thesis

Said K, Hella J, Mhalu G, Chiryankubi M, Masika E, Maroa T, et al. Diagnostic delay and associated factors among patients with pulmonary tuberculosis in Dar es Salaam, Tanzania. Infect Dis Poverty. 2017;6(1):64.

Leuenberger A, Nassoro T, **Said K**, Fenner L, Sikalengo G, Letang E, et al. Assessing stool quantities generated by three specific Kato-Katz thick smear templates employed in different settings. Infect Dis Poverty. 2016;5(1):58.

Abbreviations

AFB	Acid fast bacilli
Ag	Antigen
AIDS	Acquired immune-deficiency syndrome
aOR	Adjusted Odds Ratio
ART	Anti-retroviral therapy
BCG	Bacillus-Calmette-Guérin
BMI	Body mass index
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
CMI	Cell mediated immunity
DHS	Demographic and Health Survey
DOT	Directly observed therapy
EKNZ	Ethical Committee of North-western and Central Switzerland
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extra Pulmonary Tuberculosis
FBC	Full Blood Count
GIS	Geographical Information System
GPS	Geographical Positioning System
Hb	Hemoglobin
HIV	Human immunodeficiency virus
ICF	Informed Consent Form
IFN-γ	Interferon-gamma
IGRA	Interferon-gamma release assay
IHI	Ifakara Health Institute
INH	Isoniazid
IPT	Isoniazid preventive therapy
IQR	Interquartile range
IRB	Institutional Review Board
MDA	Mass Drug Administration
MDR-TB	Multi-drug resistant tuberculosis
MoHSW	Ministry of Health and Social Welfare
MRDT	Malaria Rapid Diagnostic Test
MTBC	*Mycobacterium tuberculosis* complex
MUAC	Mid-upper arm circumference
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
NTD	Neglected Tropical Disease
NTLP	National Tuberculosis and Leprosy Program
ODK	Open Data Kit
OR	Odds ratio
POC-CCA	Point-of-Care Circulating Cathodic Antigen
PTB	Pulmonary Tuberculosis

Abbreviations

QFT	QuantiFERON-TB Gold
RCH	Reproductive and Child Health
SD	Standard deviation
SES	Socio-economic status
sTfR	Soluble Transferrin Receptor
STH	Soil Transmitted Helminths
Swiss TPH	Swiss Tropical and Public Health Institute
TACAIDS	Tanzania Commission for AIDS
TB	Tuberculosis
TDHS	Tanzania Demographic and Health Survey
TDHS-MIS	Tanzania Demographic and Health Survey and Malaria Indicator Survey
TFNC	Tanzania Food and Nutrition Centre
TST	Tuberculin skin test
UN	United Nations
UNICEF	United Nations Children Fund
VAD	Vitamin A deficiency
VAS	Vitamin A supplementation
WHO	World Health Organization

Acknowledgements

I thank God, the almighty for this wonderful journey of attaining my PhD.

The thesis would not have been possible without the great support of many people. To my loving mother Halima Saeed, my father Said Ibrahim, my sisters, my brother and their loving families for their unbound comfort, love and understanding during the time I was undertaking this PhD. Thank you so much.

A special acknowledgment to my Faculty Representative, Prof. Marcel Tanner, for believing in me and his willingness to take me on. You have been a tremendous mentor. I am immensely grateful for the opportunity of having worked with you. Many thanks to Prof. Lukas Fenner, who has been phenomenal in his guidance and tireless support. I am grateful to Prof. Christoph Hatz who kindly agreed to act as an external examiner for my thesis.

I would also like to thank the other members of the PhD committee: Professor Karim Manji, Professor Jürg Utzinger, Dr. Anna M. Mandalakas, Dr. Nicole Ritz who have always been there when needed the most.

At the Ifakara Health Institute, I thank Dr. Salim Abdulla, Dr.Honorati Masanja and the training unit. My colleagues Nahya, a sister and special friend, Abdallah Mkopi and the amazing R4S team (Alwisa, Grace Mwangoka, Kafuruki, Michael, Omar, Rahima, Tutu). Also a lot of thanks to Mohamed, Beverly, Ali, Francis, Ida, Ali Hamad, Grace, Mlacha and Jerry, a dear colleague who deserves special thanks.

I also acknowledge the great guidance and support I have received throughout the time of this PhD, from one of the greatest childhood TB expert, Prof. Ben Marais whose advice fine-tuned this work.
The hard work was done by a brilliant TB team at Temeke and Bagamoyo; Sharifa who facilitated all administrative issues, Hosiana, Maroa, Mwajabu, Neema, Shedrack and Zainab for working so hard to assist with all the clinical work. Tito, Joseph, Kombo, Yohana and Lusuva who worked tirelessly in the field. Thanks to Lujeko and Vero for all the laboratory work. You really did an amazing job.

This work was greatly supported by at Swiss TPH by Prof. Sebastien Gagneux, I acknowledge a very supportive environment offered by the TB Research Team under your leadership and colleagues from the team; Sonia, Mireia, Andrej, Lili, Miriam, Chloe, Sebastian, Castro, Monica, Daniella and Peter. Dr. Christian Schindler and Steffi Knopp. To Dr. Klaus Reither, thanks for your valuable support. Thanks also to Damien from the Immunology unit, the Helminth drug discovery and GIS groups. Very special thanks to an amazing and loving lady, Ms. Christine Mensch, ―you are extraordinary!

Acknowledgement

I should also acknowledge the support from: Ms. Andrea Delpho at the University of Basel and the ESKAS team; the National Tuberculosis and Leprosy Control Program; Upendo Mwingira program manager at the Neglected Tropical Disease Control Program and her team; Tanzania Nutrition and Food Centre; National Aids Control Programme; National Malaria Control Program and Ester Mbutolwe of the National Institute for Medical Research.

I am grateful to many people working with District Medical office and NTLP at Temeke District and MKIKUTE who made my work possible. I have benefited so much more from their experience and commitment. Dr. Rakesh Solanki, Dr. Furaha, Dr. Fatma, Magreth, Faiza, Haji, late dada Siwa, Swalehe. Moreover, I am grateful to Leah the coordinator at Passada-Temeke.

Last but by no means least thanks to everyone who encouraged me, offered support in one way or the other during the time of my training: My grandparents Bibi Asha and Babu J, Brig. Farrah and Zainab, Mariam Bayumi and her husband Jimmy Schulte, mama Mwamy, Fatma Kabangu, mama Mwanana, Margareth Yunge, Ahmad Kalala, Alu Kalala and her husband Prof. Julius Nyang'oro. My Swiss family, Shamim and Stefan and their lovely kids Diya and Dayana. And, a very dear friend, Zena.

I greatly appreciate enormous support, love and laugher I shared with family and friends: Aminatha, Amne, Edna, Feisal, Farida, Bi Fauzia, Hadija Farrah, Hawa, Immaculata, Mahmoud, Saidi Kipoto, Mariam, Bi Sania, Peter, Rahma, Salum, Samah and Zahra.

Summary

Background: About 1.5 billion people are infected with helminth infection. The World Health Organization (WHO) estimates one third of the world population to have latent tuberculosis infection (LTBI) following exposure to an individual with smear-positive pulmonary tuberculosis (TB). Geographical distribution of these diseases and others such as human immunodeficiency virus (HIV) infection overlaps significantly in settings with inadequate sanitation, overcrowding and low socioeconomic status. Children less than five years of age (under-five) experience the highest burden of infectious disease that significantly contributes to their morbidity and mortality. Helminthiases and tuberculosis (TB) are among diseases affecting this population. Once inside the host, helminth parasites impair immune response against other infectious agents such as *Mycobacterium tuberculosis*. Helminth also negatively impact nutritional status. They compete for nutrients with their host or cause malabsorption. Consequently, the host will experience loss of appetite, reduction of nutritional intake and nutritional deficiency due to increase in the body demand for micronutrients. However, sometimes micronutrient deficiency occurs in developing countries due to marginal diets.

To tackle helminthiases, Tanzania adopted the WHO initiative to integrate preventive chemotherapy into its neglected tropical diseases control program, which also covers helminthiasis since 2009. The WHO also recommends Isoniazid Preventive Therapy (IPT) in children under-five to prevent progression of TB infection to active TB disease following exposure. To date, there are no universal guidelines for preventive chemotherapy for various helminth infections in children under-five; the focus is school-aged children and adults.

Objectives: The overall goal of this PhD thesis was to assess the interplay of helminth and tuberculosis co-infection and its impact on clinical outcomes, nutritional status, growth and cognitive development among children under-five with and without documented exposure to infectious smear-positive adult pulmonary TB case in Temeke District, Dar es Salaam, Tanzania.

Summary

The specific objectives of the thesis were; First, to determine the prevalence and intensity of helminth infections in children under-five with and without documented TB exposure in their households, and identify risk factors related to helminth infection; Second, to assess the prevalence of latent TB infection and determine its associated factors among children under-five comparing children with and without known TB exposure in their households (measured by Interferon gamma release assays (IGRAs) using QuantiFERON-TB Gold (QFT)). Last, to assess the association of helminth and *M. tuberculosis* infection and their impact on growth development (measured by WHO Z-scores), micronutrients status and cognitive function among children under-five exposed and unexposed to individuals with smear-positive pulmonary TB.

Methods: The field work for this PhD study was carried out in Temeke District, in Dar es Salaam Tanzania between October 2015 and September 2016. We combined cross-sectional and longitudinal study designs to meet our objectives. We prospectively enrolled children aged 6-59 months from households with at least an adult with smear-positive pulmonary TB, controls were recruited from households without a known TB case. We used a cross-sectional design to assess the prevalence of helminth infection and its risk factors (Objective 1). A longitudinal study design was used to evaluate the prevalence of LTBI and its associated factors after six months of follow-up comparing children with and without known TB exposure (Objective 2). To meet our third objective, we used a longitudinal study design to assess the association of helminth and *M. tuberculosis* coinfection and their impact on child growth development, micronutrients status and cognitive function. We enrolled 310 children aged 6-59 months and collected sociodemographic, socioeconomic and clinical data. We collected blood to screen for malaria, HIV, TB and analyze for micronutrients and inflammatory markers; stool and urine to screen for helminths and induced sputum samples to screen TB.

Results: The prevalence of helminth infection, particularly *Schistosoma mansoni*, was high. This high prevalence was not associated with commonly reported risk factors such as age, sex and socioeconomic

status. We report high prevalence of anemia among our study population. Overall, eight in twelve children were found to be anemic. The prevalence of anemia was higher among helminth infected children than their peers; nine out of twelve children with helminth infection were anemic.

TB screening showed equal proportion of LTBI among under-fives with and without documented exposure to infectious TB case. A small proportion of children developed active TB disease in the six month of study observation. Uptake of IPT has not reached the WHO target of 90%. About 8 in eleven children eligible for preventive therapy were reported to have been started on medication.

We found vitamin A deficiency to be prevalent; four out of twelve children were deficient. The prevalence of anemia was four times that of serum ferritin deficiency. There was no increase in serum ferritin and soluble transferrin receptor levels at the end of six-month study observation. We observed improvement of cognitive function that we could associate with better nutrition and deworming.

Conclusions: Tanzania was able to reach the millennium development goal of reducing child mortality attributing disease preventive interventions and improved social economic status. Yet the prevalence of infections that significantly contribute to under-five morbidity and mortality remains high especially in urban settings where there is better access to health services. Our findings call for further action to develop integrated guidelines to intervene the high prevalence of *S. mansoni* infection, latent TB infection due to non-household transmission, micronutrient deficiency and reduced cognitive function in under-fives. It is likely that, interventions such as micronutrient supplementation and deworming reduce helminth burden and anemia prevalence consequently improving children's development and cognition. In order to avoid lifelong consequences, interventions should aim at the first 1,000 days of life for optimal outcome.

1. Introduction

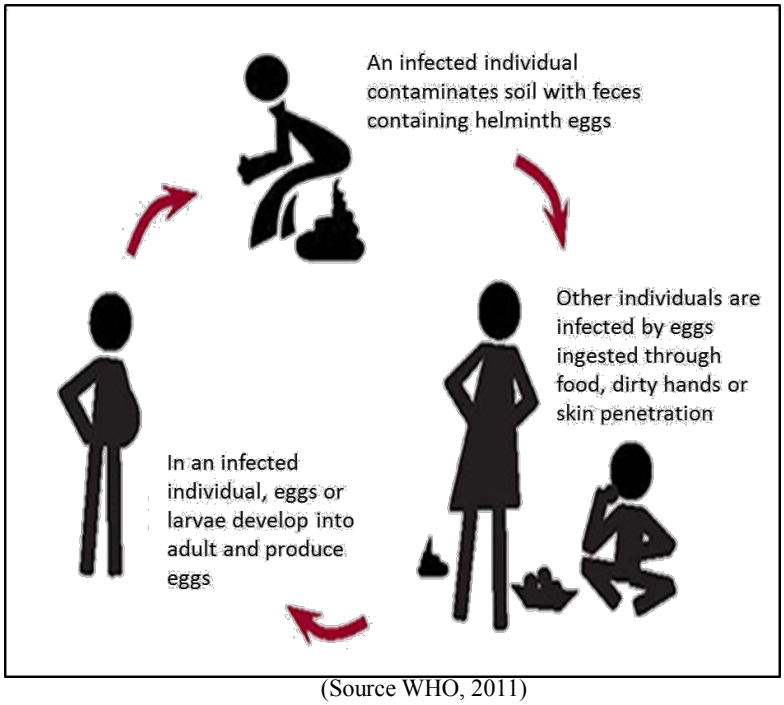
The thesis was done with aims of contributing to the understanding of helminth and *Mycobacterium tuberculosis* co-infections and their impact on clinical outcomes, growth and cognitive function in the most vulnerable children population of less than five years of age. Results from this study may help to improve guidelines on child clinical care in the future. This first chapter provides life circle of helminth, pathogenesis of *M. tuberculosis*, an overview of the burden from the two infections, the interactions of the co-infecting organisms and interventions that are made at country level and globally.

1.1. Helminth Infection in Under-five Children

1.1.1. Life cycles of Intestinal Parasites

I. Soil-Transmitted Helminth

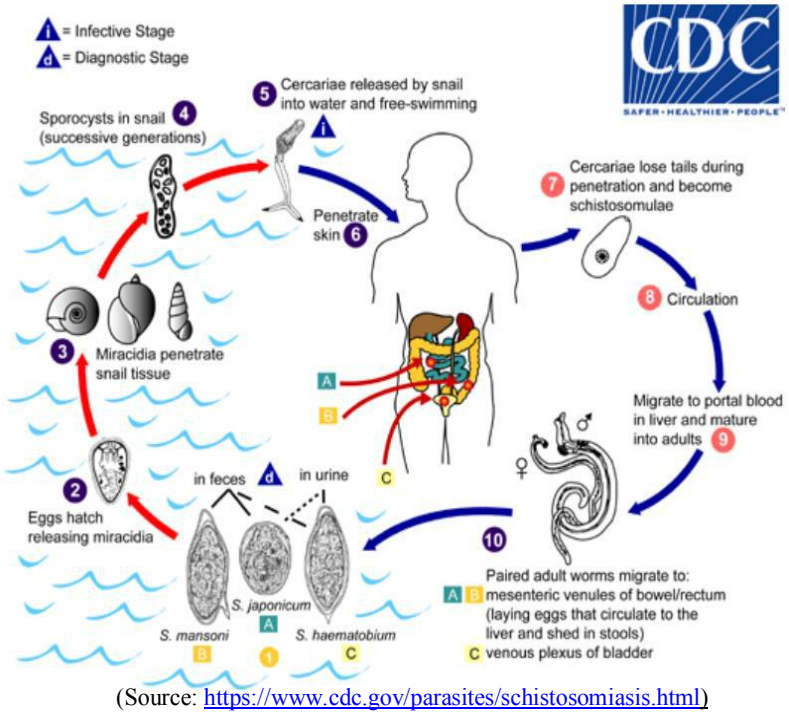
Soil-transmitted helminths (STH) are a group of nematodes causing infection worldwide and mostly affect economically deprived population. STH include *Ascaris lumbricoides*, hookworm (*Necator americanus* and *Ancylostoma duodenale*) and *Trichuris trichiura*. Transmission of STH is high in areas with inadequate sanitation, overcrowding, and low socioeconomic status and takes place without involving an intermediate host (Hotez et al., 2007, Bartram and Cairncross, 2010). Humans become infected through penetration of the skin by infective hookworm larvae in contaminated soil or by ingesting infected eggs of *A. lumbricoides* and *T. trichiura* (Bethony et al., 2006). As shown in Figure 1, human ingest eggs that hatch to larvae in the stomach and migrate to inhabit the intestines where they mature to adult worms. Infective hookworm larvae penetrate the skin to infect its host causing ―ground itch. The larvae migrate into the lungs through the bloodstream and swallowed in the esophagus to the intestines through the stomach. In the intestines, soil transmitted helminths feed on food content from its host, while hookworm sucks blood for survival. Adult worms produce large number of eggs which are released through feces into the environment to continue the circle.



(Source WHO, 2011)

Figure 1. Schematic life cycle of soil transmitted helminth

II. Schistosomiasis



(Source: https://www.cdc.gov/parasites/schistosomiasis.html)

Figure 2. Schematic life cycle of *Schistosoma* species

Eggs are excreted in human feces or urine (Figure 2, ①). Under favorable conditions the eggs hatch and release miracidia (②), which infect a suitable snail host (③). In the snail, *Schistosoma* undergoes asexual replication, sporocyst stage (④) eventually shedding cercariae into water (⑤). *Schistosoma* cercariae infect human host through skin penetration (⑥). In human host, cercarie transforms into schistosomulae (⑦) and migrate to portal vein of the liver through the bloodstream where they grow into adult worms (⑧, ⑨). Female and male adult worms unite to a couple and mate (⑩). The female shade eggs some of which are excreted in urine or feces (①) to continue the life circle.

1.1.2. Global Burden of Helminth

Over a third of the world population is infected with one or more helminth with *A. lumbricoides, T. trichiura* and hookworm being the commonest gastrointestinal infections (Borkow and Bentwich, 2000, Mccarty et al., 2014, Mejia Torres et al., 2014). Infections are widely distributed in China, Sub-Saharan Africa, East Asia and the Americas where there is enormous poverty, overcrowding and inadequate sanitation (Mishra et al., 2014, WHO, 2017c). The World Health Organization (WHO) estimates over 270 million children under the age of five years (under-five) live in these areas where intense helminth transmission occurs (WHO, 2017c). Helminth infections, though rarely fatal, cause considerable morbidity (Lustigman et al., 2012, Craig and Scott, 2014). Disease due to helminth infections continue to pose a threat to community health (Hotez et al., 2008).

Morbidity due to helminth infections is related to intensity of parasites infection (Anderson and May, 1991). Light infection may not present with symptoms, heavy infection is likely to present with general malaise, weakness and intestinal manifestations such as diarrhea and abdominal pain. Chronic helminth infection can cause malnutrition, growth, physical and cognitive development impairment (Crompton, 1984, Sakti et al., 1999, Hesham Al-Mekhlafi et al., 2008, Al-Mekhlafi et al., 2008). This is particularly the case in developing countries where children carry high infection intensity and have marginal diets (Stephenson et al., 2000). Evidence suggests that serum levels of a number of micronutrients including

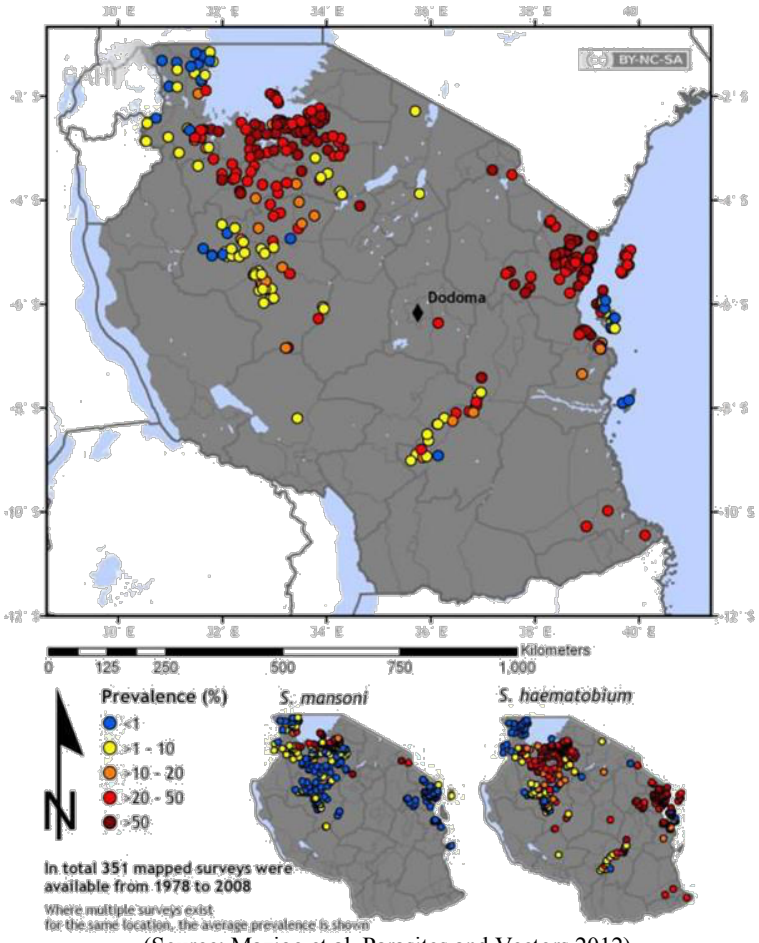
vitamin A, iron, copper, selenium, cobalt and zinc are reduced following helminth infection (Culha and Sangün, 2007, Strunz et al., 2016). Complications due to helminth infections are also species-specific: hookworm feed on iron and cause intestinal blood loss and anemia (Casmo et al., 2014), *Schistosoma haematobium* infect the urinary bladder resulting to hematuria and subsequently lead to anemia (Righetti et al., 2013, Casmo et al., 2014), while heavy *A. lumbricoides* infection lowers levels of vitamin A in its host (Sommer, 2008).

Most studies on helminth report prevalence, infection burden and complications of helminth among school-aged populations while children under-five in highly endemic areas have also shown high infection rates (Davis et al., 2014, Alemu et al., 2016). Studies have reported varying prevalence of helminth among children under-fives. A community-based cross-sectional study in Nairobi Kenya reported equal prevalence of soil-transmitted helminth among preschool-aged children and school-aged children (Davis et al., 2014). Heterogeneity of helminth prevalence rates across rural and urban settings have been reported in Kenya, where rural infection rates were generally higher than urban rates (Walson et al., 2010). A study from Ethiopia reported *S. mansoni* prevalence of 11% and that of *A. lumbricoides* was 19% (Alemu et al., 2016). In the recent years, advocacy for helminth control has been huge with involvement of international organization such as the WHO and other stakeholders (Hotez et al., 2007, WHO and Carter Centre, 2008, London Declaration, 2012, Hotez et al., 2017). In 2008, the WHO and stakeholders restored hope of eliminating neglected diseases that continue to affect many, mostly marginalized communities, by setting an ambitious goal to reach 100% anthelmintic drug coverage by 2012 in endemic countries (WHO and Carter Centre, 2008). The London Declaration on Neglected Tropical Diseases that was signed by donors, several governments, technical agencies and pharmaceutical companies committing to regularly treat 75% of children in need of anthelminthic and achieve 75% anthelminthic coverage in endemic countries as a roadmap for control and eliminate STH, schistosomiasis and other neglected tropical diseases (London Declaration, 2012).

1.1.3. Burden of Helminth Infections in Tanzania

Tanzania is among the countries with high burden of helminth infections. A prevalence of up to 85% was reported in 2000 (Bundy et al., 2000). Estimate of up to 78% of helminth among school-aged children was reported in Unguja (Knopp, 2011). A study in two district hospitals at Amana and Mwananyamala in 2003-04 in the city of Dar es Salaam reported STH prevalence of 48% among males and 52% among female children under-five attending pediatrics clinics at the two hospitals (Kalison and Mwambete, 2006). Another study in Dar es Salaam reported prevalence of 48% among children co-infected with Human immunodeficiency virus (HIV) (Mwambete et al., 2013). A recent study in Dar es Salaam among adults reported helminth prevalence of 30% from the same district where this PhD is focusing on (Mhimbira et al., 2017).

Two Schistosoma species *Schistosoma mansoni* and *Schistosoma haematobium* are known to be prevalent in Tanzania. The Coastal regions have been reporting high transmission of *S. haematobium*; the Lake Zones high transmission of *S. mansoni* shown in Figure 3 (Mazigo et al., 2012). As highlighted earlier, the transmission of schistosomiasis requires presence of suitable snail as intermediate hosts. In Tanzania, *Biomphalaria* spp, snails suitable for S. mansoni transmission are present along the lake zones and *Bulinus* spp. present along the coast. In the northern region, around Lake Victoria, *S. mansoni* prevalence of 80% among preschool-aged children was reported (Ruganuza et al., 2015). A recent study in Dar es Salaam conducted among school-age children reported a prevalence of *S. haematobium* to be 1.2% and no *S. mansoni* was reported (Mwakitalu et al., 2014).



(Source: Mazigo et al. Parasites and Vectors 2012)

Figure 3. Distribution of *S. mansoni* and *S. haematobium* in Tanzania

1.1.4. Diagnosis of Helminth Infections

Over the years, the WHO has been recommending methods such as Kato-Katz, Baermann and FLOTAC to diagnose STH, *S. Mansoni* and *S. japonicum*. Diagnosis of *S. haematobium* is made by subjecting urine sample to a filtration method for *S. haematobium* egg counts. However, Kato-Katz, Baermann and FLOTAC methods have several technical limitations and drawbacks. Kato-Katz which is a low-cost test is widely used in low-resource settings with limited human and financial capacity has low sensitivity for detection of low intensity infections (Katz et al., 1972, Booth et al., 2003, Coulibaly et al., 2013). The recently developed antigen tests such as urine-based point-of-care circulating cathodic antigens test (POC-CCA) have shown high sensitivity in diagnosing *Schistosoma* infections (Coulibaly et al., 2011). The test has been widely validated in *Schistosoma* endemic countries (Colley et al., 2013).

In a study done in Côte d'Ivoire were Kato-Katz and POC-CC were used to diagnose *S. mansoni* infection, the two tests showed varying sensitivities. Kato-Katz was reported to have low sensitivity in diagnosing infection while that of POC-CCA was >80% and no cross reactivity with *S. haematobium* was reported (Coulibaly et al., 2011). However, a recent review reported low specificity of POC-CCA suggesting cross-reactivity with *S. haematobium* infection (Ochodo et al., 2015).

1.1.5. Control of Helminth Infection in children

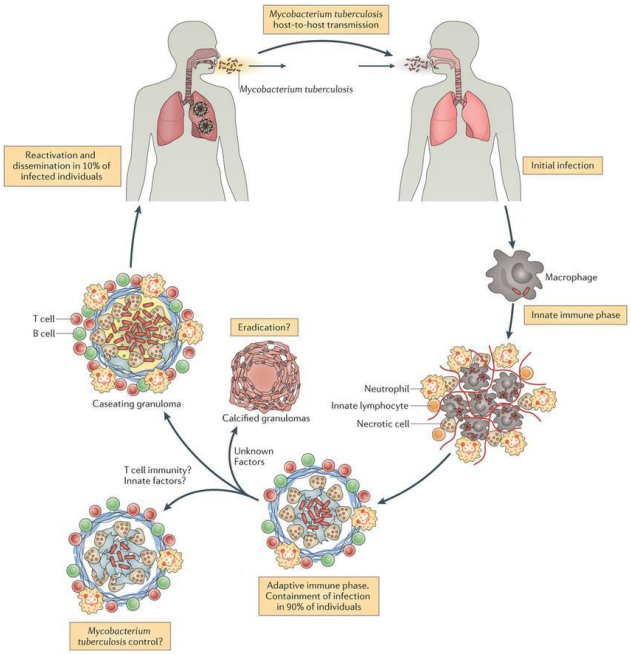
Deworming, health education and access to clean and safe water have been shown to be cost-effective in controlling helminth infections (Hotez et al., 2007). Until recently, community-wide mass drug administration (MDA) campaigns against helminth were mainly focusing on school-aged children, while preschool-aged children are also in need of appropriate preventive interventions (Davis et al., 2014). In 2008, the WHO recommended inclusion of children under the age of five years in the MDAs in setting where helminth prevalence is high. Ongoing MDAs in endemic countries started to include children under the age of five years. In Tanzania, the Neglected Tropical Disease (NTD) program focuses on school-aged and adults, while Tanzania Food and Nutrition Centre (TFNC) coordinates under-five deworming which is carried out twice a year. In addition, the TFNC includes vitamin A supplementation in under-five deworming program. Helminth infection is reduced following successful treatment to levels that cannot be detected by commonly used parasitological techniques. Nevertheless, the level of evidence on effects of deworming on height, weight, hemoglobin levels, school attendance, cognition and mortality has been shown to be low (Taylor-Robinson et al., 2012, Awasthi et al., 2013).

1.2. Tuberculosis

1.2.1. Pathogenesis of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is an acid-fast bacilli bacterium that belongs in the *Mycobacterium tuberculosis* complex (MTBC). The organism has been known to cause tuberculosis in humans since ancient times (Smith, 2003). *M. tuberculosis* contained in aerosol droplets of a person with active TB disease is transmitted to uninfected person when coughing, sneezing or talking (Pai et al., 2016). The aerosol droplets are inhaled and reach the alveoli of the lungs (Figure 4). Alveolar macrophages ingest the bacteria

and destroy or inhibit majority of these (Smith, 2003). A small number may multiply and are released when alveolar macrophages dies. If alive, the released bacteria may spread through the lymphatic system or blood stream to other parts of the body such as the bones, central nervous system, and the heart. In high risk population, the bacteria can progress and start causing clinical manifestation resulting into pulmonary tuberculosis disease or spread through the blood circulatory system to cause an extrapulmonary disease in other body sites.



(Nune-Alves et al., Nature Rev Microbiology 2014)

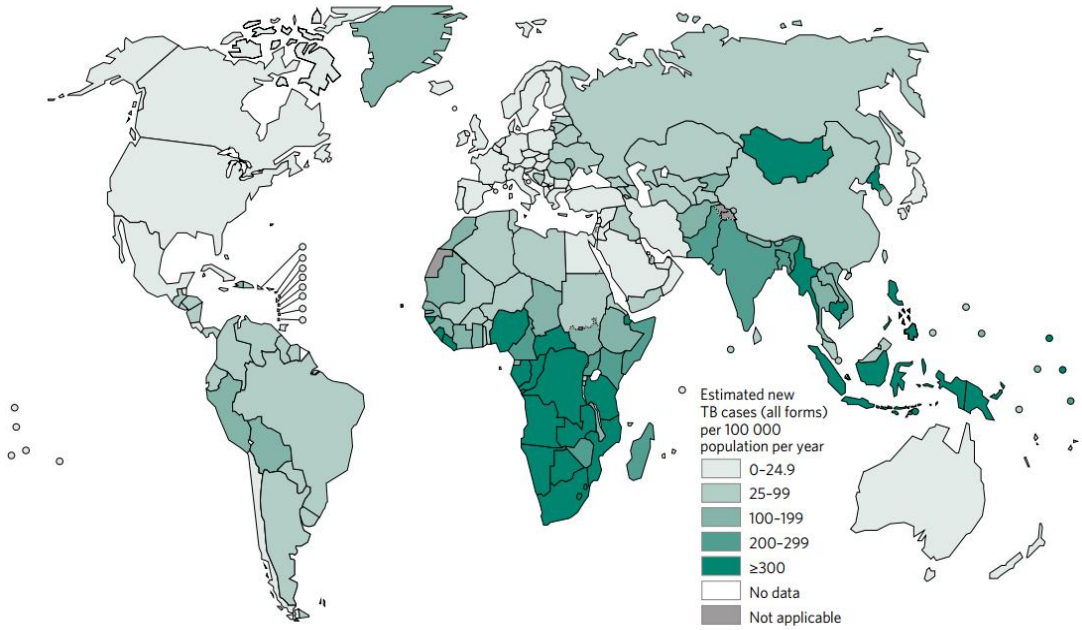
Figure 4. Pathogenesis of *Mycobacterium tuberculosis*

1.2.2. Global Burden of Tuberculosis

An estimated 10.4 million incident TB cases were reported worldwide in 2015. The disease caused 1.4 million TB related deaths in the year 2015 (WHO, 2016a). Over half of the notified TB cases were from six high TB burden countries namely; China, India, Indonesia, Nigeria, Pakistan and South Africa. Most of the high burden countries are in sub Saharan Africa and the Asia. Figure 5 shows the estimated number of new TB cases per 100,000 populations per year in 2015.

1.2.3. *Mycobacterium* Infection and Childhood Tuberculosis

Children, and particularly infants, are at an increased risk of infections. Tuberculosis remains a great challenge among children and has recently been shown to be a top ten cause of mortality in children under-five years of age (Dodd et al., 2017). Of the estimated 10.4 million incident TB cases reported in 2015, 1 million (10%) cases were among children (WHO, 2016a). It is estimated that one third of the world population is infected with *M. tuberculosis* following exposure to an infectious TB case and are asymptomatic harboring latent TB infection (LTBI) (Lule et al., 2015).



(Source: Global tuberculosis 2015 report, 2016)

Figure 5. World map showing global TB burden estimates

Risk factors for LTBI include absence of Bacillus Calmette Guérin (BCG) vaccination, index case being parent of the child, smear positivity, presence of cavities in the lungs on chest radiograph, a lesion in the upper left lobe and cough at the time of diagnosis of the index case (Gessner et al., 1998, Hu et al., 2013). Among individuals with LTBI, 5-15% may progress to an active TB disease over months or years. The risk of progressing to an active disease is especially high among infants and highest in the absence of any intervention such as isoniazid preventive therapy (IPT) (Gessner et al., 1998, Marais et al., 2006). A study among children of 1 month to 14 years in the US, a low TB burden country, reported 25% of children with history of exposure to smear-positive adult TB patients to have developed *M. tuberculosis* infection and 10% progressed to an active disease (Gessner et al., 1998). In another study in the US, 12% of children aged five years or less reported to have been exposed to individuals with smear-positive TB developed active TB in the 2 years of follow-up (Diel et al., 2011). These children were not treated with IPT. In high burden countries, children exposed to individuals with smear-positive TB had 30-50% risk of being infected with *M. tuberculosis* and developing the disease at a later stage (Lienhardt et al., 2003, Nakaoka et al., 2006, Nguyen et al., 2009, Jaganath et al., 2013). Several factors have been identified to play a role in the progression of LTBI to active TB disease; these risk factors include helminth infection, HIV infection, poor nutritional status, indoor air pollution, poor housing and parent's level of education (Marais et al., 2004, Elias et al., 2006, Lonnroth et al., 2009, Desalu et al., 2013).

1.2.4. Burden of Childhood Tuberculosis in Tanzania

Tanzania is among the 30 countries with high tuberculosis burden with an estimated incidence of 300 per 100,000 population (WHO, 2016a). Childhood tuberculosis still is a public health concern in the country. The National Tuberculosis and Leprosy Control Program (NTLP) reported childhood TB cases to have contributed 10% of the total cases reported in the 2000-2010. NTLP emphasizes on adult case management for disease control purposes and recently produced a new guideline for management of childhood tuberculosis giving age specific recommendations (NTLP and MoHSW, 2012). There is no childhood multidrug resistance tuberculosis (MDR TB) estimates given by the program.

1.2.5. Contact Tracing and Prevention of Tuberculosis in Children

Several studies have reported children typically acquire *M. tuberculosis* most likely from their parents/caregivers and relatives who live with them in the same households (Jaganath et al., 2013, Ritz and Curtis, 2014, Lule et al., 2015). This has led to the WHO and stakeholders develop guidelines that recommend contact screening for children living with an infectious smear-positive pulmonary TB case as key strategy in controlling TB among this young population. NTLP guideline recommends tracing and evaluating all children in contact with infectious TB case (NTLP and MoHSW, 2012). If resources allow, tuberculin skin test (TST) is recommended to be included in TB screening and its interpretation is dependent on the immune status of the child (NTLP and MoHSW, 2012). In HIV positive children, a TST induration of >5mm in the absence of active TB disease is considered diagnostic for LTBI. TST is not recommended for screening HIV positive children older than 12 months (NTLP and MoHSW, 2012).

Due to limited resources, TB screening in Tanzania is mostly symptom based. In evaluating children contacts, a detailed medical history and thorough physical examination are recommended. AntiTB medication is immediately recommended for children found to have the disease based on scoring under the country's direct observed treatment (DOT). Once TB disease has been ruled out, isoniazid preventive therapy (IPT) is initiated irrespective of immune status. Antiretroviral therapy (ART) is recommended not to delay IPT and vice versa. In addition, the program also recommends source case investigation if a child is diagnosed with TB without prior documentation of a source case.

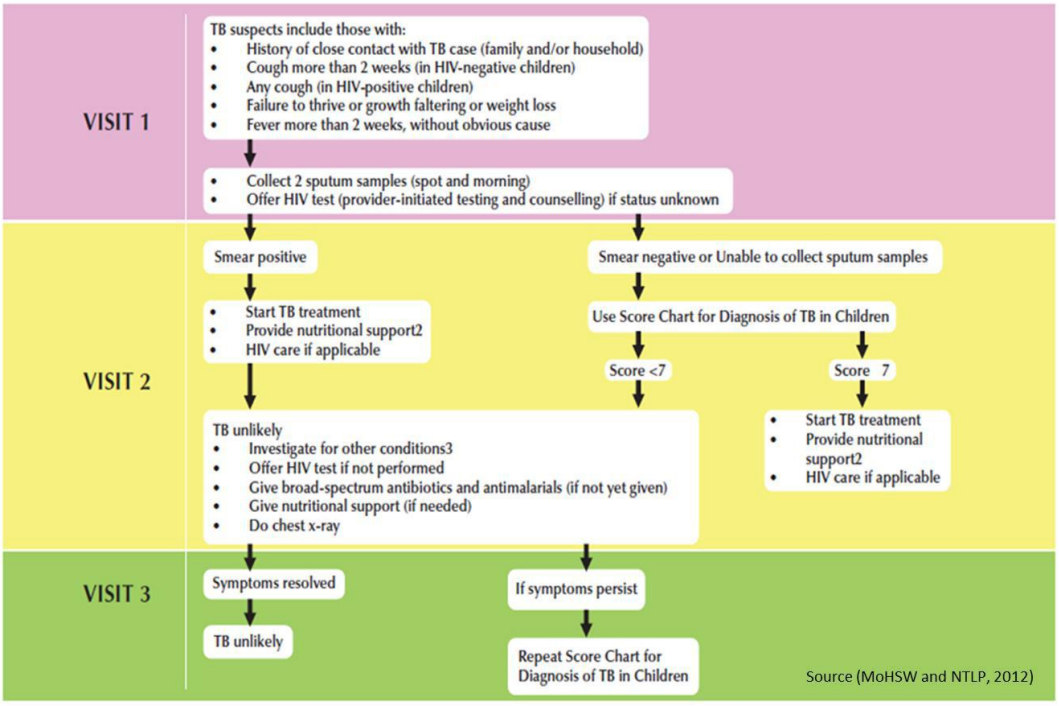
1.2.6. Diagnosis and Treatment of Tuberculosis in Children

Similar to other under-resourced countries, Tanzania is faced with difficulty in the diagnosis of childhood tuberculosis. Still hugely relies on the use of microscopy and few sites with Xpert MTB/RIF machines especially in urban settings (MoHSW, 2013a, NTLP and MoHSW, 2015). With majority of TB patients in the major cities, diagnostic challenges are not different to rural settings of the country given the high numbers of residents and scarcity of advanced diagnostics. Due to paucibacillary nature of childhood TB, bacteriological confirmation is not possible in many childhood TB cases (Marais and Graham, 2014).

Other children may present with extrapulmonary disease whose diagnosis is also challenging. To improve bacteriological confirmation of childhood TB, high quality multiple samples from the appropriate source are required. Yet, this has also not been achieved in many high TB burden settings. The lack of facilities and advanced diagnostics for childhood cases pose a threat and has led to underreporting of TB cases among children.

The Tanzanian NTLP guideline recommends BCG vaccine to prevent TB in children. The vaccine is administered to all infants regardless of their HIV status except those whose mothers have TB at the time of birth and those who present with suggestive symptoms. BCG is delayed until antiTB or two weeks after IPT completion (NTLP and MoHSW, 2012). Despite the vaccine, TB-infected children are at high risk of progressing to active TB particularly severe forms of disease such as TB meningitis and disseminated disease. The country's NTLP emphasize on symptomatic TB screening and a TB scoring chart which includes: i) medical history; ii) history of TB contact; iii) clinical findings; iv) HIV test for all TB suspects; v) tuberculin skin test whenever available; vi) bacteriologic confirmation whenever possible and vii) other relevant investigations such as chest radiography for pulmonary TB if suspected and fine needle aspiration/spinal radiograph/abdominal ultrasound if extrapulmonary TB is suspected (Figure 6) (NTLP and MoHSW, 2012).

For effective control of TB disease in high risk group of young children, there is an urgent need to consistently screen for *M. tuberculosis* infection and initiate preventive treatment to reduce active TB disease incidence. Most TB programs in low income and high TB-incidence rates use tuberculin skin test (TST) instead of interferon gamma release assays (IGRA), such as the ELISpot-based test (T-SPOT.TB) and enzyme-linked immunosorbent assay (ELISA)-based test QuantiFERON-TB Gold (QFT), that are restricted to research use only (Shah et al., 2011, Rose et al., 2012, Mandalakas et al., 2015).



1. Antibiotics should treat common causes of chest bacterial infections. Consider continuing with other investigations if child has already received antibiotics without improvement. Avoid aminoglycosides and fluoroquinolones, as they have anti-TB effects
2. Nutritional support may include nutritional counselling, provision of supplemental or therapeutic feeds
3. Other conditions might include cardiac disease, congenital lung disease, fungal infections (including pneumocystis pneumonia), and chronic lung diseases such as asthma or bronchiectasis, parasitic infections, or oncologic disease
4. Antibiotics should treat common causes of chest bacterial infections. Consider continuing with other investigations if child has already received antibiotics without improvement. Avoid aminoglycosides and fluoroquinolones, as they have anti-TB effects
5. Nutritional support may include nutritional counselling, provision of supplemental or therapeutic feeds
6. Other conditions might include cardiac disease, congenital lung disease, fungal infections (including pneumocystis pneumonia), and chronic lung diseases such as asthma or bronchiectasis, parasitic infections, or oncologic disease

Figure 6. Algorithm for diagnosis pulmonary TB in children under six years of age

Although TST is recommended in screening for *M. tuberculosis* infection, its interpretation is questioned in populations where BCG is widely given and non-tuberculous mycobacteria (NTM) are prevalent (Pérez-Porcuna et al., 2014). QFT one of the IGRAs was approved for *M. tuberculosis* infection screening by the Food and Drug Administration in the USA in 2005 which recommends a negative QFT in recently TB exposed children <5 years to be repeated after 8-10 weeks to identify recent test conversions and resolve indeterminate results (CDC, 2010). Unlike TST, IGRAs offer improved specificity because most of the NTM do not harbor proteins that stimulate T-cell production and have less cross-reactivity with BCG vaccination (Pai et al., 2007, CDC, 2010). However, IGRAs results may also be influenced by other

factors such as other live attenuated immunizations, malnutrition, young age, HIV co-infection and helminth, however all these interactions are understudied (Hesseling and Mandalakas, 2011). WHO recommendations regarding IGRAs to diagnose TB for routine clinical care in middle- and low-income countries, including in children have not been positive outside of research settings due to lack of clear evidence in improved diagnostic accuracy for IGRAs above TST (Hesseling and Mandalakas, 2011). In recent years, research groups have put forward initiatives to develop new diagnosis tools that can diagnose both TB infection and disease accurately. This has resulted to immunodiagnostic assays other than IGRAs (Chegou et al., 2012). This shows persistent need for novel antigens with much higher discriminatory power to differentiate between *M. tuberculosis* infection and active TB. Additionally, total RNA (tRNA) from whole blood has recently been demonstrated from a large study in TB endemic settings as a biomarker in discriminating latent TB infection from an active TB disease (Anderson et al., 2014). Due to the limitations of IGRAs in high-incident countries, a quantified exposure score was recently proposed to serve as a surrogate measure of *M. tuberculosis* infection, specifically in TB-exposed children (Morrison et al., 2008, Hill and Ota, 2010, Mandalakas et al., 2012, Mandalakas et al., 2015).

1.3. Tuberculosis and Helminth Co-infection

1.3.1. Epidemiology of Tuberculosis and Helminth Co-infection

Africa is faced with highest challenge of overlap of diseases of poverty as shown in Figure 7, the burden of helminthiases is high in settings with inadequate sanitation, overcrowding, and low socioeconomic status; the same characteristics that govern transmission of TB, HIV and malaria are endemic (Hotez et al., 2007, Bartram and Cairncross, 2010, Sachiyo Nagi, 2013, Salgame et al., 2013, Mhimbira et al., 2017).

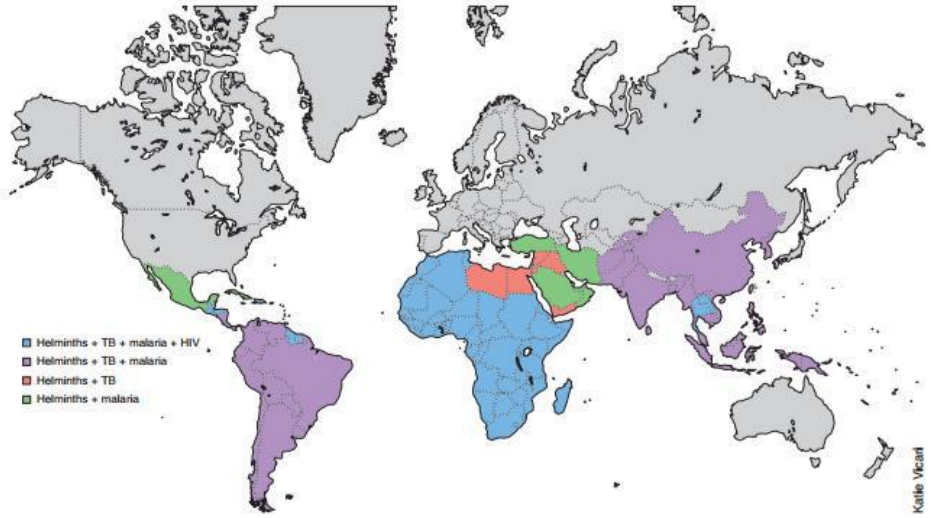


Figure 7. World map showing the geographic distribution of helminth and TB, malaria and/or HIV co-infection in adults

1.3.2. Immune Modulation by Helminth Infection

Large body of evidence suggests helminth and *M. tuberculosis* interacts at the level of the immune system (Resende Co et al., 2007, Salgame et al., 2013). Pre-existing helminth infection has been shown to induce production of $CD4^+$ T-helper 2 cells immune pathway that impedes $CD4^+$ T-helper 1 cell development. The $CD4^+$ T- helper 1 cells are the involved in body protection against invading pathogens such *M. tuberculosis* (Salgame et al., 2013, Mishra et al., 2014, Babu and Nutman, 2016). These mechanisms act in synergy to produce a helminth-modulated immuno-regulatory environment that compromises Th1 and Th17 responses and favors T regulatory cell activities that impinges host resistance against infections including as *M. tuberculosis*, HIV and malaria (Figure 5) which increases host's susceptibility to developing active TB or HIV disease (Resende Co et al., 2007, Rafi et al., 2012, Salgame et al., 2013). In a study in Brazil, higher prevalence of intestinal helminth, predominantly *Strongyloides stercoralis*, was reported among adult TB patients compared to non-TB control group (Tristao-Sa et al., 2002). A study in Kenya reported the odds of hookworm in school-aged children with LTBI 3 times higher than in children without hookworm, indicating an association between the two (Sachiyo Nagi, 2013). A recent study by in Tanzania showed *S. mansoni* to be associated with TB disease (Mhimbira et al., 2017).

Chronic intestinal helminths have also been shown to compromise efficacy of BCG vaccines. There have also been reports on the effect of helminth on other vaccines such as tetanus, HIV and malaria vaccines (Resende Co et al., 2007, Markus and Fincham, 2007, Mcsorley and Maizels, 2012). Intestinal helminth infections have an effect on IGRA and TST performance. A study in Bangladeshi reported increased proportion of indeterminate QFT among children with helminth infection compared to those without helminth infection (Thomas et al., 2010). In a study in Venezuela, statistically significant relationship between TST-positive children and helminths infection was reported, with high odds ratio of 3.5 between *Trichuris trichiura* and TST positive results to TST-negative results (Verhagen et al., 2012). However, reports on tuberculosis and intestinal helminths co-infection among children under the age of five years in the fastest growing city of Dar es Salaam are scarce.

1.4. Rationale and Hypotheses

The Tanzanian NTD program carries MDA in collaboration with its partners since the country has high prevalence of helminth (WHO, 2011b). The National Institute for Medical Research (NIMR) includes helminth research among its priorities of 2013-2018. However, many studies in the country focus on school-aged children (Mwakitalu et al., 2014). Most studies on *Schistosoma* infection among preschool–aged using urine-based diagnostic test are done in high risk areas; Point-of-care circulating cathodic antigen (POC-CCA) test has never been used for diagnosis of Schistosoma along the coastal regions such as Dar es Salaam (Ruganuza et al., 2015). According to the WHO report of 2016, TB remains to be a public health concern in Tanzania. The country had 62000 notified TB cases in 2015 (WHO, 2016a). Dar es Salaam reports 22% of all notified cases in the country. The documented co-existence of helminthiasis and TB puts children at an increased risk of both infections. The WHO advocates the health system to prevent missed opportunity as a way of achieving zero TB deaths in children under-fives (WHO, 2013). Little is known about the impact of helminth and TB co-infection on clinical outcomes, nutritional status and cognitive function among children under the age of five years with and without documented TB exposure since for many years the focus has been on school-aged children. Therefore, it is important to study the impact of helminth

infection in this vulnerable population to define the true burden of helminth and coinfection with TB to design specific interventions needed to improve growth, development and cognitive function of children under-five.

This PhD thesis has the following hypotheses:

1. Is the prevalence of helminth infection among under-fives in particularly urban settings as high as that reported among school-aged children in the same setting to justify mass treatment programs?
2. Are children under five years of age in Dar es Salam faced with non-household transmission of TB? and
3. Are there differences in growth, cognitive performance, nutritional status and micronutrient levels among children younger than five years of age with helminth and TB co-infection and without co-infection?

1.5. Objectives

The overall objective of this PhD thesis was to assess the prevalence and intensity of helminths infections as well as TB co-infection among children under-five years of age with and without documented exposure to infectious smear-positive adult TB cases, and its impact on clinical outcomes, nutritional status, cognitive development in Temeke District, Dar es Salaam, Tanzania. The study took advantage of the ongoing cohort study of adult TB patients and their controls (TB-Dar) in the same district.

The following specific objectives were also defined:

1. To determine the prevalence and intensity of helminth infections in under-fives exposed and not exposed to TB cases in households, and identify risk factors such as age, sex, and characteristics of caregivers;
2. To assess the prevalence of latent TB infection and determine risk factors among under-fives comparing children with and without documented exposure to infectious TB cases in the household as measured by IGRAs using QFT assays; and
3. To assess the association of helminth and *M. tuberculosis* co-infections and their impact on child growth (measured by WHO Z-scores), micronutrients status and cognitive function among children under-five exposed and not exposed to individuals with smear-positive TB, considering additional factors such as HIV and malaria co-infections.

1.6. Materials and methods

1.6.1. Study setting and Study design

The study was conducted in Temeke District in Dar es Salaam, Tanzania. Dar es Salaam. Dar es Salaam is a business capital of Tanzania and home to approximately 5 million people, about 10% of the country's population (National Bureau of Statistics and Office of Chief Goverment Statistician, 2013). Temeke district one of the three districts in Dar es Salaam. It is densely populated district with a population of 1,369,000 and average household size of 3.9 according to the 2012 census report (National Bureau of Statistics and Office of Chief Goverment Statistician, 2013). In 2014 the district had 4400 notified TB cases (NTLP and MoHSW, 2015). Temeke district has malaria prevalence of approximately 10% with seasonal variations. Recent helminth prevalence among adults was reported to be 30% (Mhimbira et al., 2017). Dar es Salaam is among MDA regions in Tanzania with recent MDA campaign among under-fives carried out in June 2017 (MoHSW, 2017).

We combined cross-sectional and longitudinal study designs to meet our objectives. A cohort of children under-five with and without documented exposure to infectious TB cases was prospectively recruited. A cross-sectional design was used to study the prevalence of helminth infection and its associated risk factors (aim 1). A longitudinal study design was used to assess the prevalence of latent tuberculosis infection and identify risk factors for *M. tuberculosis* infection among children under-five comparing those with and without known TB exposure (aim 2). To meet our third objective, we also used a longitudinal study design where we assessed the effect of TB and helminth co-infection on micronutrients, growth and cognitive development.

This PhD study took advantage of an ongoing funded population-based cohort study of smear-positive adult TB patients and their household controls in Temeke (TB-Dar) (Mhimbira et al., 2017).

1.6.2. Study population

Inclusion criteria for the study were (i) age 6-59 months; (ii) living in Temeke district and likely to remain in the study area for at least 12 months; (iii) whose parents/caretaker have read and signed a written informed consent; (iv) children documented to have exposure to a smear-positive TB case within their households for cases and (v) for controls, children from a household with no documentation of a TB case (as confirmed by a standard questionnaire).

1.6.3. Sample Size

The sample size was calculated based on the assumption that the true prevalence for intestinal helminth infection among under-fives to be 30%. This sample size was expected to estimate local helminth prevalence with a precision of 5% and an error probability of 5%.

1.6.4. Study procedures

Before commencement of the study, we trained the research team on the study protocol, data collection tools, case report forms and study's Standard Operating Procedure (SOP). The team comprised of research medical doctor, clinical officers, study nurses, laboratory personnel, field officers and research assistants. Individuals with smear-positive TB aged >15 years with children aged 6-59 months in their households were identified at National Tuberculosis and Leprosy Program (NTLP) TB clinic at Temeke hospital. Identified adults were regarded as index and asked to bring their children for screening in accordance with NTLP guideline. Field workers visited household with TB index cases to recruit under-fives and recorded geographic coordinates using a hand-held GPS device. Using study's SOP for control recruitment (Appendix 1: Standard Operating Procedure: Invitation of control children), the field worker identified a neighboring household with a child 6-59 month old and ascertained if there was any person aged 15 years or more who was on antiTB treatment (Grimes and Schulz, 2005). If there was none, the house was considered ―TB free‖ and invited the under-five and his/her parent to the Reproductive and Child Health (RCH) Clinic at Temeke Hospital. Upon arrival, parents/caretakers were given informed consent form (ICF) and given a minimum of one and half hour to read.

Parents/caretakers who could not read were asked to bring an impartial witness who assisted them to read and witnessed signing on the ICF. Parents/caregivers who agreed to the study information as written on the ICF were taken to see the study doctor to consent. Parents/caregivers who were able to read were asked to sign the ICF; parents/caregivers who were not able to read and write were asked to put a thumbprint in the presence of their witnesses. Children who met study criteria were enrolled and study procedures followed according to Figure 8 below. A brief description of study procedure is given below, and more details are given in subsequent chapters. All study procedures were done according to SOPs approved by the Ifakara Health Institute's Quality Assurance team.

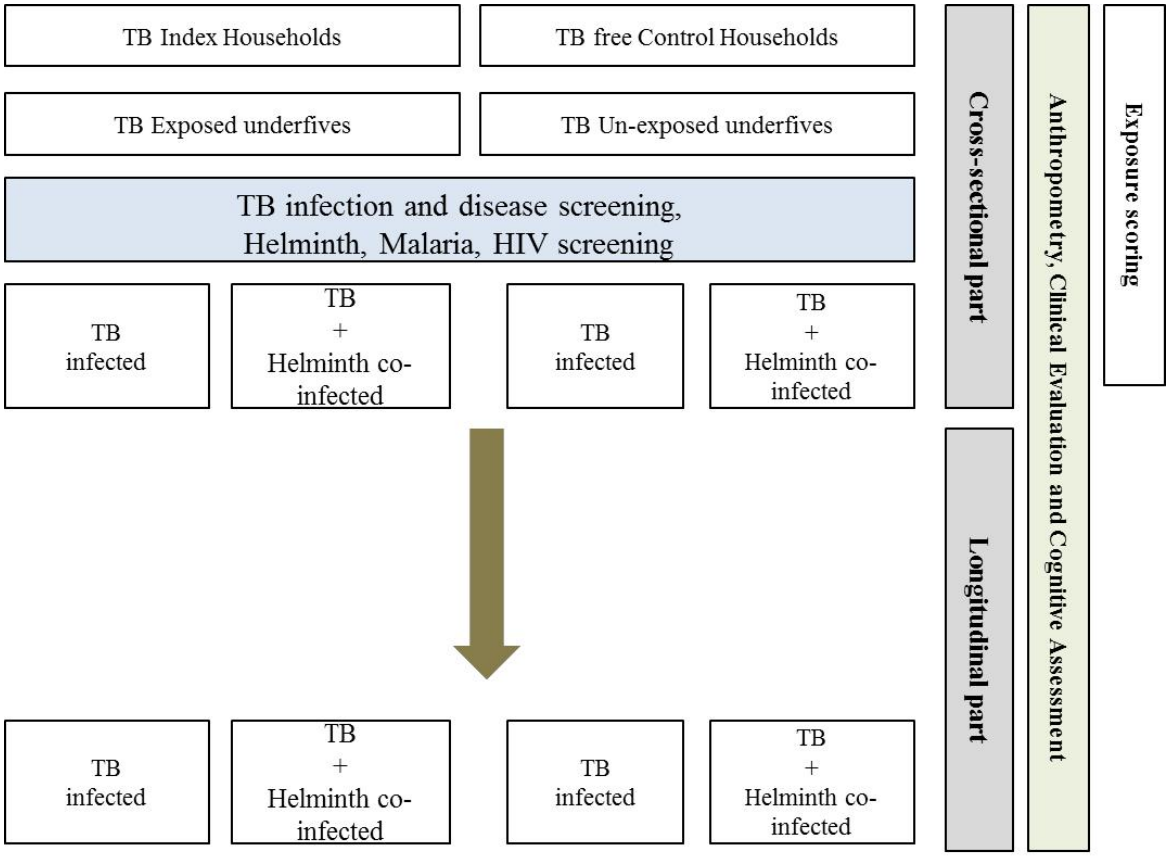


Figure 8. Schematic diagram of study procedures

***Clinical procedures*:** At enrollment, we systematically collected the following information using a structured questionnaire with the following variables; socio-demographic, socioeconomic status of parents/caregivers, immunization history, medication history including IPT and antihelminthics, clinical

history, co-morbidities such as HIV of both the child and the mother (Table 1). We recorded anthropometric parameters of all children and computed appropriate WHO Z-scores (standard deviation scores) to assess child growth and nutritional status. We assessed cognitive development using an adopted standardized questionnaire from Malawi (Gladstone et al., 2010). Clinical evaluation was repeated at three and six month follow-up visits.

***Cognitive assessment*:** We used a validated Malawi Development Assessment Tool (MDAT) to assess children's development and cognition (Gladstone et al., 2010). The assessment tool was translated into Kiswahili and verified by a Kiswahili-speaking expert. A medical doctor with expert training in pediatrics (Mcdonald et al., 2013) trained the study nurses before commencing the study. Monthly refresher trainings were conducted on site for the duration of the study. Trained study nurses assessed each child for 40 minutes and recorded scores. Parents or caregivers of acutely ill children were advised to return within a week of the child's recovery for re-assessment (Mcdonald et al., 2013). Cognitive assessment was done at enrollment and six month follow-up visit.

***TB exposure score assessment*:** We assessed TB exposure at enrollment using a score by Mandalakas et al (Mandalakas et al., 2012). The score has 10 questions with binary responses with a score of 0-10 that takes into consideration a number of aspects of TB exposure that includes; sleep proximity and maternal TB (questions 1-4), infectivity of the index case (questions 5-7), frequency of the child's exposure to the index case (questions 8-9) and question 10 which quantifies the number of adults with TB in the household. The ten questions in the TB exposure score are: (i) is the child's mother the index case?; (ii) is the child's primary caregiver the index case?; (iii) does the child sleep on the same bed as the index case?; (iv) does the child sleep in the same room as the index case?; (v) is the index case coughing?; (vi) is the index case a pulmonary TB case?; (vii) does the index case have smear-positive TB?; (viii) does the index case live in the household with the child?; (ix) does the index case see the child every day?; and (x) is there more than one TB case in the household? Control participants were not assessed for TB exposure.

Materials and methods

Table 1. Overview of clinical and laboratory data collected during the study

Variable	Visit	
	Baseline	Follow-up
Socio-demographic (child)		
Informant, age (months), sex, area of residence, GPS coordinates	X	
Weight (kg), height (cm)	X	X
Socio-demographic (caretaker/parents)		
Level of education, occupation, history of previous residency	X	
Mobile numbers of parent/caretaker (personal, neighbors)	X	
Household characteristics (SES)		
Cigarette smoking, source of water, type of toilet, cooking fuel, household food security, electricity, type of flooring, number of rooms, household possessions	X	
Household characteristics (additional for helminths infection)		
If drinking water is boiled, fruits and vegetable consumption, food handling hygiene, travel history within the last six months	X	X
HIV-specific variables (if HIV positive)		
CTC card number, HIV-clinic attending ,date of diagnosis	X	
CD4 cell count, viral load, Antiretroviral regimen and start date	X	
Immunization history (with date of immunization)		
BCG, OPV/DPT-HB-Hib, rotavirus, measles	X	X
Nutritional history		
Breast-fed/bottle-fed, duration of breast-fed, age at weaning, appetite	X	X
Food, vegetable and fruit consumption	X	X
Illnesses, medication and hospitalization		
Past illnesses or hospitalization (TB, malaria, helminths, diarrhea, respiratory infections)	X	X
Treatment prescribed (including IPT) during each illness/hospitalization	X	X
TB index specific variables (from NTLP card)		
NTLP card number, TB treatment regimen, start/stop dates	X	
Sputum smear result, Drug resistance (if done)	X	
TB exposure score (from TB index household)		
If the mother is the index source, primary care giver	X	
Sleeps in the same bed/room as child	X	
Lives in the same household as child, in daily contact with child, If index presented with cough	X	
Pulmonary TB present, individuals with smear-positive sputum	X	
More than one index in household	X	
Laboratory variables		
Hemoglobin level, ferritin level, sTfR, CRP, AGP, Vitamin A, HIV test (PCR/Rapid), malaria RDT, QFT [1]	X	X
Helminths type and intensity	X	**(X)**
Smear results, Xpert MTB/Rif (if TB suspected)	X	**(X)**

[1] QFT was repeated at three month

(X) Repeated at six month visit

AGP: Alpha 1 glycoprotein; BCG: Bacillus Calmette–Guérin; CRP: C-reactive protein; DPT-HB-Hib: Diphtheria, pertussis, tetanus-Hepatitis B-*Haemophilus influenza* type b; GPS: Geographical positioning system: HIV: Human immunodeficiency virus; IPT: isoniazid preventive therapy; OPV: Oral polio vaccine; PCR: Polymerase chain reaction; sTfR: Soluble transferrin receptor; TB: Tuberculosis

***TB infection and Active TB diseases screening*:** We used a comprehensive symptom-based TB screening tool to screen for active TB disease. At baseline, in addition to symptom-based screening, we performed chest X-rays for each child and Xpert MTB/RIF. At follow-up visits, we used the symptom-based screening tool; X-ray and/or Xpert MTB/RIF were ordered only if there was an indication. Confirmation and final diagnosis were based on clinical evaluation, chest X-ray findings and Xpert MTB/RIF, according to Graham et al (Graham et al., 2012).

***Blood collection*:** We collected blood, urine, a scotch tape, stool samples and induced sputum from all children (Table 2). The blood samples were collected under special instructions for various analysis intended for the study. Blood samples for zinc measurement were collected at least 12 hours after the last meal and just before breakfast. Venous blood was drawn using zinc-free blood collection needles into serum tube, followed by EDTA tube, QFT tubes and lastly in S-Monovette heparinized zinc-free tubes. Blood sample in S-Monovette heparinized zinc-free tubes was delivered to the lab within 30 minutes of collection where it was immediately centrifuged and plasma was transferred using plastic zinc-free pipet into zinc-free tubes and frozen at -20°C until analysis.

1.6.5. Data collection

We designed questionnaires according to accepted standards, piloted and modified where appropriate. We collected data as shown in the overview Table 1 above. Data were directly entered electronically into tablets computers using the open-source software open data kit (ODK; http://opendatakit.org/) (Steiner et al., 2016). Data were saved in a password protected database and back-ups were performed regularly.

1.6.6. Laboratory procedures

Laboratory procedures were done at the Bagamoyo Research and Training Centre (BRTC), Ifakara Health Institute (IHI) and in collaborating laboratories at Temeke Municipal Hospital in Dar es Salaam, Human Nutrition Laboratory at ETH Zurich University in Zurich and VitMin Lab, Willstaett, Germany. Table 2 below summarizes the tests done on samples. The detailed laboratory procedures are covered in the respective chapters of this thesis.

Materials and methods

Table 2. Specimen collected, and tests done during the study

Sample	Laboratory test(s)
Stool	Kato-Katz, Baermann and FLOTAC methods for detection of helminth infections
Urine	Circulating Cathodic Antigen for detection of *S. mansoni* Urine filtration for detection of *S. haematobium*
Anal scotch tape	Direct microscopy for *Enterobius vermicularis* diagnosis
Blood	Full blood count (FBC), malaria, QuantiFERON-TB Gold, Human Immunodeficiency Virus (HIV) test, Vitamin A, Serum ferritin, Soluble transferrin receptor, Zinc
Sputum	Xpert MTB/RIF assay

2. *Schistosoma,* other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania

Khadija Said [1,2,3] ♠, Jerry Hella [1,2,3], Stefanie Knopp [2,3], Tatu Nassoro [1], Neema Shija [1], Fatma Aziz [4], Francis Mhimbira [1,2,3], Christian Schindler [2,3], Upendo Mwingira [5,6], Anna M. Mandalakas [7], Karim Manji [8], Marcel Tanner [2,3], Jürg Utzinger [2,3], Lukas Fenner [9] ♠

1 Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania, **2** Swiss Tropical and Public Health Institute, Basel, Switzerland, **3** University of Basel, Basel, Switzerland, **4** Temeke Municipal Council Hospital, Dar es Salaam, Tanzania, **5** Neglected Tropical Disease Programme, Dar es Salaam, Tanzania, **6** National Institute for Medical Research (NIMR), Dar es Salaam, Tanzania, **7** The Global Tuberculosis Program, Texas Children's Hospital, Baylor College of Medicine, Houston, United States of America, **8** Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, **9** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

♠E-mails: ksaid@ihi.or.tz (KS) and lukas.fenner@ispm.unibe.ch (LF)

Short title: Helminths in preschool-aged children in urban Tanzania

Manuscript I

2.1 Abstract

Background: Despite the high prevalence of helminth infections among preschool-aged children, control programs in sub-Saharan countries primarily focus on school-aged populations. We assessed the prevalence of helminth infections and determined risk factors for infection among preschool-aged children in the urban setting of Dar es Salaam, Tanzania.

Methodology: Starting in October 2015, we conducted a 12-month prospective study among tuberculosis (TB)-exposed children under the age of 5 and unexposed controls from neighboring households. At the time of recruitment, we collected medical histories, assessed development and cognitive functions, and performed medical examinations. We performed full blood cell counts and screened for HIV and malaria. Point-of-care circulating cathodic antigen (POC-CCA), urine filtration, Kato-Katz, FLOTAC, and Baermann tests were employed to detect helminth infections in urine and stool. Helminth infections were stratified for *Schistosoma* and other helminths to identify risk factors, using logistic regression.

Principal findings: We included 310 children with a median age of 26 months (inter quartile range 17–42) in the study. Among these, 189 were TB-exposed and 121 TB-unexposed. Two thirds of the children were anemic (hemoglobin level <11 g/dL) and the HIV prevalence was 1.3%. *Schistosoma* spp. were the predominant helminth species (16.0%; 95% confidence interval [CI] 12.1–20.3). Other helminth infections were less frequent (9.0%, 95% CI 6.3–12.8%). Poor hygiene, use of household water sources, and TB-exposure were not associated with helminth infection. Development and cognitive scores did not significantly differ in helminth-infected and uninfected peers, but hemoglobin levels were significantly lower in helminth-infected children (10.1g/dl *vs.* 10.4 g/dl, p=0.027).

Conclusion/significance: In Dar es Salaam, a city with more than 4 million, the prevalence of *Schistosoma* infection among preschool-aged children was high. Setting-specific interventions that target preschool-aged children and urban settlements should be considered to reduce the transmission of *Schistosoma* and other helminth infections and to improve children's health.

Manuscript I

Author Summary

In many African countries, children under the age of 5 years are at considerable risk of acquiring parasitic worm infections. Yet, most of the neglected tropical disease control programs in Africa do not include preschool-aged children in deworming campaigns. Chronic parasitic worm infections may impair children's growth and their cognitive development. We conducted a 12-month prospective study of children younger than five years in the Temeke district, Dar es Salaam — the economic capital of Tanzania— to assess the prevalence of parasitic worm infection. Among 310 included children, we found that one in six children was infected with the blood fluke *Schistosoma*, while one in 11children were infected with soil-transmitted helminths. Anemia was found among 65% of children, particularly among those infected with parasitic worms. The high prevalence of *Schistosoma* infection in this urban setting, despite improved water supply and sanitation as well as limited open freshwater contact shows the pressing need to identify parasitic worm hotspots in urban areas. Setting-specific interventions targeting preschool-aged children and urban settlements, among others, should be considered to reduce the transmission of Schistosomiasis and other parasitic worm infections.

2.2. Introduction

Helminth infections affect more than 1.5 billion people globally and are particularly common amongst economically deprived populations (Utzinger et al., 2012, Pullan et al., 2014). The burden of helminthiases is high in settings with inadequate sanitation, overcrowding, and low socioeconomic status; the same characteristics that govern transmission of tuberculosis (TB) (Hotez et al., 2007, Bartram and Cairncross, 2010, Sachiyo Nagi, 2013, Mhimbira et al., 2017). Helminth infections, though rarely fatal, cause considerable morbidity (Lustigman et al., 2012, Craig and Scott, 2014). In children, heavy intensity helminth infections can impair physical growth and cognitive development, and lead to micronutrient deficiencies and anemia (Jukes et al., 2002, Hotez et al., 2007). Subsequently, if anemia and its underlying causes are not managed, it may lead to death in children with additional co-morbidities (Albonico et al., 2008, Scott et al., 2014). Children with poor cognitive development have difficulties learning and perform poorly at school, thereby failing to reach their full potential (Berhe et al., 2009). Chronic helminth infection is also detrimental to the functioning of the immune response against infectious diseases such as TB and, hence, increases the risk of developing TB in later life (Dinardo et al., 2016). Associations between TB and helminth infections have been reported for school-aged and adult populations (Tristao-Sa et al., 2002, Sachiyo Nagi, 2013).

Children living in resource-constrained areas in sub-Saharan Africa and elsewhere are at high risk of acquiring helminth infections, given their poor hygienic environments and unattended outdoor access when playing with peers. Early detection and effective management of helminth infection can improve children's health and wellbeing. Most studies of helminth infections have focused on school-aged populations, though preschool-aged children in highly endemic areas might also show high infection rates (Alemu et al., 2016). For example, a community-based, cross-sectional survey conducted in Nairobi found that the soil-transmitted helminth prevalence among preschool-aged children was similar to that of school-aged children (Davis et al., 2014).

In 2008, the World Health Organization (WHO) set an ambitious goal to reach 100% anthelmintic drug coverage by 2012 in endemic countries (WHO and Carter Centre, 2008). Yet, the WHO did not include preschool-aged children in targeted deworming campaigns until 2008.

In 2009, Tanzania adopted the WHO initiative to integrate preventive chemotherapy into its neglected tropical diseases control program, which also covers helminthiases. To date, the focus has been on school-aged children and adults (MoHSW, 2017). No universal guidelines exist for using chemotherapy to prevent various helminth infections in preschoolers. To assess the prevalence and intensity of helminth infections among preschool-aged children, including its impact on clinical outcomes, we conducted a cross-sectional survey in an urban setting in Temeke district, Dar es Salaam, Tanzania. We employed a suite of standardized, quality-controlled diagnostic methods to enhance the accuracy of species-specific helminth detection and quantification (Colley et al., 2013).

2.3. Methods

Ethics statement

The study was approved by the Institutional Review Board of the Ifakara Health Institute (reference no. IHI/IRB 12-2015), the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (reference no. NIMR/HQ/R.8a/Vol. IX/2002), and the Ethics Committee of Northwestern and Central Switzerland (reference no. EKNZ UBE-15/49). Children were enrolled after their parents or caregivers gave written informed consent.

Infections with *Schistosoma* spp. were treated with praziquantel (40 mg/kg), soil-transmitted helminths with albendazole (200 or 400 mg depending on children's age), and *Strongyloides stercoralis* with ivermectin (3 mg), immediately after diagnosis (MoHSW, 2013b). Additionally, children with a history of TB exposure without active disease were started on isoniazid preventive therapy (20 mg/kg) (NTLP and MoHSW, 2012). Children with anemia (hemoglobin <11 g/dL) were given iron or folic acid supplements, as clinically appropriate. In addition, dietary counseling was provided to parents and caregivers of all

children with impaired nutritional status. Human immunodeficiency virus (HIV)-positive children were referred to a care and treatment center for further management, in keeping with Tanzanian guidelines.

Study setting

The study was carried out in the Temeke district, Dar es Salaam, Tanzania (Mhimbira et al., 2017) between October 2015 and September 2016. The district has routine TB contact tracing in place supported by TB patients who successfully completed treatment. Mass deworming in the district is coordinated by the neglected tropical disease control coordinator. Although the local water authority supplies piped water to the district, due to the high demand, residents also use ground water sources from boreholes for household chores which is vulnerable to pollution from pit latrines. The borehole water is used by most of the residents in the district (National Bureau of Statistics and Regional Commissioner's Office, 2014).

Study design

The current manuscript used the baseline data of the case-control study pertaining to the epidemiology of TB and helminth coinfections among children exposed and not exposed to TB. Preschool-aged children were recruited from households with an adult TB case (sputum smear-positive for acid-fast bacilli) and from TB-free neighboring households (to serve as controls), based on previously described operating procedures (Grimes and Schulz, 2005). In the present cross-sectional study embedded within the aforementioned case-control study, we assessed the prevalence of helminth infections and determined associations with household characteristics, child development and cognition, and hematological factors in the survey children.

Study population and sample size

We aimed for a sample size of 308 children, aged 6–59 months, with 154 TB-exposed and 154 TB-unexposed preschool-aged children, and with one child recruited per household. This sample size would allow estimating local helminth prevalence with a precision of 5% and an error probability of 5% if the helminth prevalence were of the order of 30%.

Study procedures

Children were seen by trained study clinicians who collected sociodemographic and socioeconomic information and obtained their medical history, including prior illnesses and use of medication. Clinicians assessed children for TB signs and symptoms (NTLP and MoHSW, 2012). A TB-exposure score chart from South Africa was used to assess TB exposure (Mandalakas et al., 2012). The TB score was then categorized into (i) not likely to have TB infection (score of 1–6), or (ii) presumptively TB infected (score of ≥7). In addition, all children had a chest X-ray done. Trained study nurses recorded anthropometric measurements (height and weight), collected samples (blood, urine, stool, adhesive tape slide, and induced sputum), and performed development and cognitive assessments (gross motor, fine motor, language, and social components).

On the day of enrollment, parents or caregivers were given two empty containers labeled with the participant's unique identification number and invited to submit one fresh morning stool sample and one urine sample of their child the following day. The samples were transferred to a nearby laboratory within 3 hours of collection. Due to limited financial and human resources, only a single stool sample and urine sample could be collected. Additionally, each participant was provided with a plastic pocket that contained an adhesive tape (50 x 20 mm) and a pre-labeled glass slide and asked to submit the slide with anal adhesive tape for *Enterobius vermicularis* examination as described elsewhere (Salim et al., 2014). We collected venous blood samples for full blood cell (FBC) counts and for malaria and HIV screening, along with induced sputum samples for microbiological investigation. All samples were received at Temeke clinic, transferred to a laboratory in appropriate temperature-controlled cooler boxes, and processed within 5 hours of receipt.

Cognitive assessment

A validated Malawi Development Assessment Tool (MDAT) that was translated into Kiswahili was used to assess children's development and cognition (Gladstone et al., 2010). A medical doctor with expert training in pediatrics (Mcdonald et al., 2013) trained the study nurses before commencing the study.

Monthly refresher trainings were conducted on site for the duration of the study. Each child was assessed for 40 minutes. Parents or caregivers of acutely ill children were advised to return within a week of the child's recovery for assessment (Mcdonald et al., 2013).

Laboratory procedures

Helminth investigations: A single stool sample was obtained s from each child, subjected to triplicate Kato-Katz thick smears, and examined under a microscope by trained laboratory technicians for species-specific diagnosis of helminth infection. Triplicate Kato-Katz thick smear slides and the FLOTAC methods were employed for the diagnosis of *Ascaris lumbricoides*, hookworm, *Hymenolepis diminuta*, *Schistosoma mansoni* and *Trichuris trichiura* while the Baermann technique was used to detect larvae of *Strongyloides stercoralis* (Knopp et al., 2014). The adhesive tapes were examined under a microscope for *E. vermicularis* eggs (Salim et al., 2014). To screen for *S. haematobium* eggs, urine samples underwent urine filtration in duplicates using a hydrophilic polycarbonate membrane filter with a pore size of 20 μm (Sterlitech; Kent, United States of America) and subsequent examination of the filters for *S. haematobium* eggs. Microhematuria was examined by reagent strips (Hemastix; Siemens Healthcare Diagnostics, Eschborn, Germany). Urine samples were additionally tested for *Schistosoma* spp. antigens using a point-of-care circulating cathodic antigen (POC-CCA) cassette test (Rapid Medical Diagnostics; Pretoria, South Africa) which has been primarily validated for *S. mansoni*, but cross-reactivity has been reported (Colley et al., 2013, Ochodo et al., 2015). Using a visual aid tool and based on a semi-quantitative score, the POC-CCA results were interpreted as negative, trace, 1+, 2+, or 3+. All slides with adhesive tapes, Kato-Katz thick smears, and urine filters were stored in boxes, and 10% of the slides were re-examined for quality control purposes by experienced laboratory technicians within 6 months (Knopp et al., 2014). All helminth investigations were conducted at the Bagamoyo Research and Training Centre. The standard operating procedures have been described in detail elsewhere (Becker et al., 2016).

*Microbiological investigations***:** Xpert MTB/RIF (Cepheid; California, United States of America) was performed on induced sputum samples at the Temeke district hospital laboratory to aid in the diagnosis of

TB. The laboratory is continuously monitored for quality by the Central Tuberculosis Reference Laboratory (Dar es Salaam, Tanzania).

*Blood testing***:** Blood samples were screened for malaria with a rapid diagnostic test (Access Bio; New Jersey United States of America), and for HIV infection using Alere Determine HIV-1/2 (Alere; Massachusetts United States of America) if the child's age was ≥18 months or RNA polymerase chain reaction if <18 months. The FBC were done with an MS4 Vet hematology analyzer (Diamond Diagnostics; Massachusetts, United States of America) to determine hematological indices such as hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red blood cell distribution width (RCDW).

Data collection and definitions

Data was recorded into tablet computers, using open data kit (ODK; http://opendatakit.org/), and *odk_planner*, a data management tool. Laboratory results were entered into ODK from paper forms.

A helminth infection was defined as positive when eggs or larvae of the following species were microscopically identified: *A. lumbricoides, E. vermicularis,* hookworm, *H. diminuta, S. haematobium, S. mansoni, S. stercoralis, or T. trichiura. Subsequently,* helminth infections were grouped into (i) schistosomiasis, defined as infection with either *S. mansoni* or *S. haematobium* (based on stool microscopy, using Kato-Katz thick smears, urine filtration and/or positive POC-CCA urine test results) and (ii) other helminthiases, including infections with any of the other helminths (*A. lumbricoides, H. diminuta, hookworm, T. trichiura, E. vermicularis and S. stercoralis*). A POC-CCA test was regarded as positive if the band revealed 1+, 2+, or 3+. In sensitivity analyses, POC-CCA definition included also trace-positive results.

In the absence of any signs or symptoms suggestive of TB and/or as ascertained by Xpert MTB/RIF, a child was considered presumptively TB infected if the TB exposure score was ≥7 and unlikely to have a TB infection if the score was 1–6 (Mandalakas et al., 2012). Anemia was defined as hemoglobin <11.0 g/dl, as

per WHO recommendations (WHO, 2011a). Anthropometric z-scores were calculated using the 2006 WHO Growth Standards in Stata version 13.1 using the zscore06 command (WHO, 2006).

Statistical analysis

Absolute frequencies and proportions were used to describe children, parents/caretakers, and household characteristics overall and stratified by the two groups of helminthiases. A measure of socioeconomic status was derived from a factor analysis of household asset variables and defined as low or high for score values below and above the median, respectively. Clinical outcomes included anemia, cognitive score and anthropometric measures (weight and height). We performed mixed logistic regression analyses with random intercepts at the level of matched pairs to identify risk factors for helminth infection, considering schistosomiasis and other helminthiases. We constructed multivariable core models comprising age, sex, type of toilet, hygiene behavior, and parent education variables based on clinical relevance and added other variables as appropriate, one by one. We also performed a sensitivity analysis to identify risk factors for Schistosoma spp. infection using the core model as above and considering trace results in the POC-CCA urine cassette test as positive. We used box-plots to compare the four MDAT components in children with and without helminth infections and calculated the overall median and interquartile range (IQR) of the total MDAT score and across relevant subsamples. We dichotomized the four components of the MDAT score at their median and ran mixed logistic regressions to compare scores between helminth-infected and uninfected children. We also compared hematological indices according to the presence of helminth infections using mixed linear regression models. All analyses were performed in Stata version 13.1 (Stata Corporation; College Station, United States of America).

2.4. Results

Study flow and baseline characteristics of children

We invited 398 parents and caregivers with children aged 6–59 months to participate. Parents/caregivers of 325 children consented and their children were enrolled. Of those, 310 completed the study procedures. Eight children did not provide their sociodemographic and clinical information, six did not submit stool and urine samples for helminth diagnosis, and one parent withdrew consent (Figure 9).

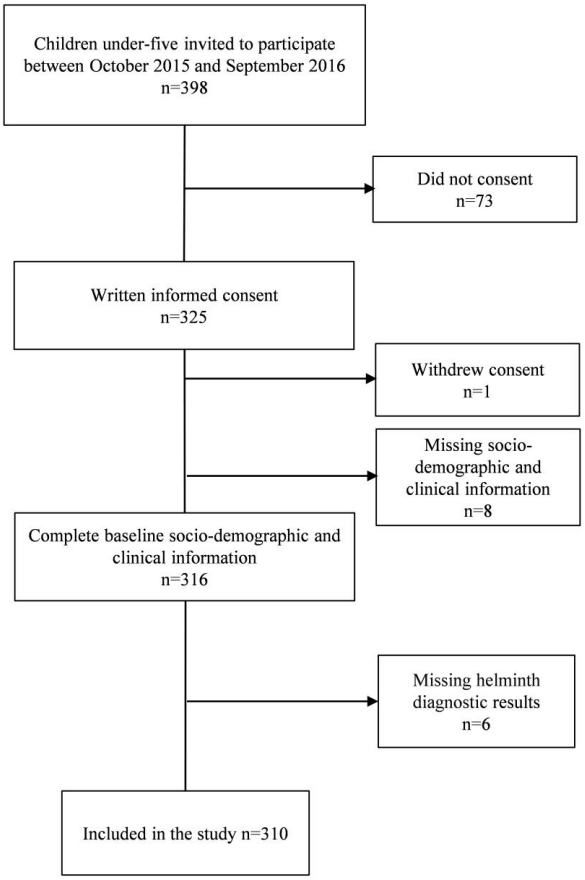


Figure 9. Flow chart of the 310 participants included in the study.

Of the 310 participating children, 160 (52%) were girls and the median age was 26 months (IQR: 17-42 months, range 6–58 months). The median height-for-age Z-score (HAZ) was -1.14 (95% confidence interval (CI): -1.91 to -0.20) (Table 3). A total of 189 (61%) children were exposed to smear-positive adult pulmonary TB patients and four (1.3%) were HIV-positive. Twenty-nine (9%) mothers reportedly tested HIV-positive during pregnancy. Fourteen (5%) children had a positive malaria rapid diagnostic test, six

(2%) reportedly received anthelmintic within the three months prior to enrollment in the study. Parents/caretakers of 23 (7%) children reported having moved from other regions to Dar es Salaam after their children were born.

Prevalence of helminth infections

The overall prevalence of *Schistosoma* spp. infection was 16.0% (95% CI 12.1–20.3%). *Schistosoma* spp. infection as determined by POC-CCA was found in 47 children (15.0%; 95% CI 11.6–19.6%), while *S. haematobium* eggs were only found in the urine of three individuals (1%) (Table 4) and no *S. mansoni* eggs were found in any of the Kato-Katz thick smears. None of the investigated children were found to be *S. mansoni*-positive by stool microscopy. There was no difference in the distribution of children with *Schistosoma* infection in young (6–24 months) and older (25–59 months) age groups (53% *vs.* 47%, p=0.3) or between boys and girls (51% *vs.* 49%, p=0.7). There was also no significant difference between TB-exposed and unexposed children (67% *vs.* 60%, p=0.3), as shown in Table 3. The prevalence of *Schistosoma* spp. infection (as determined by POC-CCA) increased to 31.0% (95% CI 26.3–36.7%) when considering trace results as positive.

The prevalence of other helminth species infections, excluding *Schistosoma* spp., was 9.0% (95% CI 6.3–12.8%). The most frequently detected helminth species was *S. stercoralis* (16 children; 5%), followed by *E. vermicularis* (6; 2%), and hookworm (6; 2%). Infections with *A. lumbricoides* and *H. diminuta* were found in only one child each, and no *T. trichiura* infection was observed (Table 4). The difference in the distribution of helminth infections between TB-exposed and unexposed children was not statistically significant (62% *vs.* 54%, p=0.4).

Five children (2%) had dual species helminth infections: two with *Schistosoma* spp.-*S. stercoralis*; and one each with *Schistosoma* spp.-*E. vermicularis*, *E. vermicularis*-hookworm, and *A. lumbricoides*-*H. diminuta*. One child had a triple species helminth infection with *Schistosoma* spp.-*E. vermicularis*-hookworm.

Manuscript I

Table 3. Baseline sociodemographic, socioeconomic, and clinical characteristics of 310 preschool-aged children enrolled between October 2015 and September 2016, and their parents/caregivers in the Temeke district, Dar es Salaam, Tanzania.

Characteristic	All (n=310)	Any helminth species [1]		*Schistosoma* spp. [2]	
		Infected (n=74)	Not infected (n=236)	Infected (n=49)	Not infected (n=261)
Child characteristics					
Age (months), median (IQR)	26 (17-42)	23 (17-36)	28 (17-43)	23 (18-38)	27 (16-42)
Age groups (months					
6-12	52 (17)	11(15)	41 (17)	8 (16)	44 (17)
13-24	92 (30)	29(39)	63 (27)	18(36)	74 (28)
25-36	71 (23)	16(22)	55 (23)	10(21)	61 (24)
37-48	57 (18)	10(14)	47 (20)	7 (14)	50 (19)
49-59	38 (12)	8 (11)	30 (13)	6 (13)	32 (12)
Sex					
Female	160(52)	37(50)	123 (52)	24(49)	136 (52)
Male	150(48)	37(50)	113 (48)	25(51)	125 (48)
Delivered by					
Caesarean section	40 (13)	13(17)	27 (11)	10(20)	30 (12)
SVD	253(20)	56(76)	197 (84)	38(76)	215 (82)
Unknown	17(5)	5(7)	12 (5)	1(2)	16 (6)
Born at gestation age (weeks)					
Pre-term <37	9 (3)	2(2)	7 (3)	1(2)	8 (3)
Term ≥37	284(92)	67(91)	217 (90)	45(96)	239 (91)
Unknown	17(5)	5(7)	12 (5)	1(2)	61 (6)
Birth weight (kg)					
Low <2.5	28(9)	5(7)	24 (10)	4(8)	25 (10)
Normal ≥2.5	265(86)	64(86)	200 (85)	44(09)	220 (84)
Unknown	17(5)	5(7)	12 (5)	1(2)	16 (6)
Immunization status					
BCG, with scar	306 (99), 260 (85)	71(96)	235 (99)	42(86)	218 (84)
Measles	263(85)	68(92)	195 (83)	46(94)	217 (83)
HIV status					
Positive	4 (1.3)	2(3)	2 (1)	0	4 (2%)
Negative	306 (98.7)	72(97)	234 (99)	49 (100)	257 (98)
Hemoglobin level (g/dL)					
Anemic <11.0	203(65)	56(76)	147 (62)	35(71)	168 (64)
Not anemic ≥11.0	104(34)	17(23)	87 (37)	13(27)	91 (35)
Missing	3 (1)	1(1)	2 (1)	1(2)	2 (1)
Malaria rapid diagnostic test					
Positive	14(5)	3(4)	11 (5)	3(6)	11 (4)
Negative	296(95)	71(96)	225 (95)	46(94)	250 (96)
TB exposure history					

Manuscript I

Characteristic	All (n=310)	Any helminth species [1]		*Schistosoma* spp. [2]	
		Infected (n=74)	Not infected (n=236)	Infected (n=49)	Not infected (n=261)
Exposed	189 (61)	46(62)	143 (61)	33(67)	156 (60)
Unexposed	121 (39)	28(38)	93 (39)	16(33)	105 (40)
TB exposure score					
Likely not infected	197 (64)	50(68)	147 (62)	33(67)	164 (63)
Likely infected	113 (36)	24(32)	89 (38)	16(33)	97 (37)
Deworming status (past 3 months)					
Not dewormed	304 (98)	72(97)	232 (98)	48(98)	256 (98)
Dewormed	6 (2)	2(3)	4 (2)	1(2)	5 (2)
HAZ-scores					
Median (IQR)	-1.14 (-1.91 to -0.2)	-1.16 (-1.72 to -0.07)	-1.12 (-1.94 to -0.33)	-1.17 (-1.58 to -0.13)	-1.09 (-1.98 to -0.31)
WAZ-score					
Median (IQR)	-1.14 (-2.07 to -0.35)	-1.3 (-2.22 to -0.28)	-1.12 (-1.99 to -0.35)	-1.34 (-2.36 to -0.69)	-1.10 (-2.05 to -0.33)
WHZ-score					
Median (IQR)	-0.94 (-2.02 to -0.13)	-1.16 (-2.02 to -0.17)	-0.79 (-1.86 to -0.13)	-1.48 (-2.07 to -0.10)	-0.75 (-1.83-15.0)
Household characteristics					
Number of people					
<6	190 (61)	43(58)	147 (62)	29(59)	161 (62)
≥6	120 (39)	31(42)	89 (38)	20(41)	100 (38)
Household income per month (USD)					
<100	108 (35)	28(38)	80 (34)	15(31)	93 (36)
≥100	202 (65)	46(62)	156 (66)	34(69)	168 (64)
Water source					
Bore well	90 (29)	15(20)	27 (11)	14(29)	28 (11)
Tap	153 (49)	43(58)	158 (67)	27(55)	174 (67)
Unknown	67 (22)	16(22)	51 (22)	8 (16)	59 (22)
Type of household toilet					
Septic tank	93 (30)	28(38)	65 (28)	21(43)	72 (28)
Pit latrine	217 (70)	46(62)	171 (72)	28(57)	189 (72)
Hygienic practices					
Poor	36 (12)	12(16)	24 (10)	7 (14)	29 (11)
Good	274 (88)	62(84)	212 (90)	42(86)	232 (89)
SES					
Low	159 (50)	40(54)	119 (50)	28(57)	131 (50)
High	151 (50)	34(46)	127 (50)	21(43)	130 (50)

Manuscript I

Parent/caregiver characteristics	All (n=310)	Any helminth species [1]		*Schistosoma* spp. [2]	
		Infected (n=74)	Not infected (n=236)	Infected (n=49)	Not infected (n=261)
Mothers prior pregnancies					
Unknown	17(5)	5(7)	12(5)	1(2)	16(6)
0	88 (30)	19(26)	69 (29)	9 (19)	79 (30)
1-2	142(48)	39(53)	103(44)	30 (64)	112(43)
≥3	63 (17)	11(15)	52 (22)	7 (15)	56 (21)
Mothers HIV status during pregnancy					
Unknown	24(8)	6(8)	18(7)	1(2)	23(9)
Positive	29(9)	4(5)	25 (11)	3(6)	26 (10)
Negative	257(83)	64(86)	193(82)	45(92)	212(81)
Mothers marital status					
Single	76 (25)	19(25)	57 (24)	14(29)	62 (24)
Married	217(70)	50(68)	167(71)	34(69)	183(70)
Unknown	17(5)	5(7)	12(5)	1(2)	16(6)
Parent education level					
No or primary education	244(79)	63(85)	181(77)	42(86)	202(77)
Secondary/higher education	66 (21)	11(15)	55 (23)	7 (14)	59 (23)
Parent occupation					
Unemployed	196(63)	49(66)	147(62)	31(63)	165(63)
Employed	114(37)	25(34)	89 (38)	18(37)	96 (37)
Family migration history since child birth					
Migrated	23(7)	8 (11)	15(6)	3(6)	20(8)
Did not migrate	189(61)	44(59)	144(61)	36(73)	153(58)
Unknown	98 (32)	22(30)	77 (33)	10(20)	88 (34)

HAZ, height for age, moderate to severe stunting (z-score≤-2); HIV, human immunodeficiency virus; TB exposure score based on Mandalakas et. al. [25]; SVD, spontaneous vaginal delivery; WAZ, weight for age, moderate to severe underweight (z-score≤-2); WHZ, weight for height, moderate to severe wasting (z-score≤-2); USD, United States dollars (1 USD=2,190 Tanzanian Shillings); SES, Socioeconomic status (low= below median of the principal asset score, high =above the median of the principal asset score)

[1] Any helminth infection defined as positive when eggs or larvae of the following species were microscopically identified: *A. lumbricoides, E. vermicularis,* hookworm, *H. diminuta, S. haematobium, S. mansoni, S. stercoralis,* or *T. trichiura*; or a positive POC-CCA test result indicating *Schistosoma* infection (test result 1+, 2+, 3+)

[2] *Schistosoma* spp. included *S. mansoni* and *S. haematobium*

Table 4. Frequency distribution of helminth species among preschool-aged children in Dar es Salam, Tanzania

Helminth infection	All n (%)	≤24 months		>24 months	
		Male	Female	Male	Female
		n (%)	n (%)	n (%)	n (%)
Total	310 (100)	72 (100)	72 (100)	78 (100)	88 (100)
Any helminth infection [1]	74 (23.9)	25(34.7)	15 (20.8)	12 (15.4)	22 (25.0)
Schistosomiasis					
***Schistosoma* spp.(POC-CCA)** [2]					
Any positive result (trace and positive)	97 (31.3)	27 (37.5)	21 (29.2)	20 (25.6)	29 (33.0)
Trace	50 (16.1)	11 (15.3)	12 (16.7)	11 (14.1)	16 (18.2)
Positive	47 (15.2)	16 (22.2)	9 (12.5)	9 (11.5)	13 (14.3)
1+	34 (11.0)	8 (11.1)	9 (12.5)	6 (7.7)	11 (12.5)
2+	12 (3.9)	8 (11.1)	0(0.0)	2 (2.6)	2 (2.3)
3+	1(0.3)	0 (0.0)	0(0.0)	1 (1.3)	0 (0.0)
Schistosoma haematobium [3]					
Positive	3 (0.97)	1(1.5)	1(1.5)	0 (0.0)	1 (1.1)
Other helminth infection [4]					
Any of the other helminth species	28 (9.0)	10(13.9)	5(6.9)	4 (5.1)	9 (10.2)
Strongyloides stercoralis	16 (5.2)	6(8.3)	3(4.2)	3 (3.9)	4 (4.6)
Enterobius vermicularis	6 (1.9)	1(1.4)	1(1.4)	1 (1.3)	3 (3.4)
Hookworm	6 (1.9)	3(4.2)	2(2.8)	0 (0.0)	1 (1.1)
Ascaris lumbricoides	1 (0.3)	0(0.0)	0(0.0)	0 (0.0)	1 (1.1)
Hymenolepis diminuta	1 (0.3)	0(0.0)	0(0.0)	0 (0.0)	1 (1.1)

[1] Any helminth was defined as positive when eggs or larvae of the following species were microscopically identified: *A. lumbricoides, E. vermicularis,* hookworm, *H. diminuta, S. haematobium, S. mansoni, S. stercoralis,* and *T. trichiura*

[2] Point-of-care circulating cathodic antigen test for detection of S*chistosoma* spp. *infection* (POC-CCA test result 1+, 2+, 3+). Cross-reactivity with *S. haematobium* cannot be fully excluded.

[3] Based on urine filtration (egg-positive urine filtration)

[4] Other helminth species (based on stool or adhesive tape microscopy): *A. lumbricoides*, *E. vermicularis*, hookworm, *H. diminuta*, and *S. stercoralis*

Five participants had dual species and one participant a triple species helminth infection

Risk factors for helminth infections

Schistosoma spp. infection was significantly associated with having a septic tank toilet in the household (adjusted odds ratio (aOR) 2.04, 95% CI: 1.02–4.07, p=0.042; Table 5). Higher education of parents/caregivers tap water at home, and better hygiene practices showed no significant association with *Schistosoma* spp. infection. Additionally, *Schistosoma* spp. infection was similar in TB-exposed and unexposed children (aOR 1.34, 95% CI: 0.67–2.68, p=0.4) (Table 5). In the sensitivity analysis that considered POC-CCA trace results as positive, none of the variables included in the core model, including having septic tank toilets, were associated with *Schistosoma* spp. infection (Supplementary Table 1). Furthermore, none of the risk factors were significantly associated with any of the other helminth infection,

including having a septic tank toilet (aOR 0.92, 95% CI: 0.35–2.40, p=0.9) (Table 5).

Association of helminth infections with development and cognitive scores

The overall median MDAT score in the study population was 3.30 (IQR 2.78–3.49). There was no significant difference in the overall median cognitive score in helminth-infected and uninfected children (3.20 [95% CI 2.74–3.44] *vs.* 3.33 [95% CI 2.80–3.50], p=0.2 (Supplementary Table 2)). There was also no effect of *Schistosoma* spp. infection on the overall median cognitive score among the two groups (3.17 [95% CI 2.78–3.44] *vs.* 3.32 [95% CI 2.78–3.50], p=0.2).

The median gross motor score tended to be higher among preschool-aged children with a helminth infection compared to their uninfected peers. The median fine motor (0.79 *vs.* 0.83), social (0.85 *vs.* 0.89), and language scores (0.86 *vs.* 0.88) tended to be lower among helminth-infected compared to helminth-uninfected children (Figure 10), but none of the differences achieved statistical significance.

Association of helminth infection with hematological parameters

Almost two-third of the children (203; 65%) were anemic; nine (4%) of those with anemia had a positive rapid malaria diagnostic test result. Moderate anemia (hemoglobin level 7.0±9.9 g/dl) was most prevalent (49%), while mild anemia (hemoglobin 10.0±10.9 g/dl) was found in 44%, and severe anemia (hemoglobin <7 g/dl) was found in 14 of the anemic children (7%). Five (6%) children with mild anemia, three (3%) with moderate anemia, and one (7%) with severe anemia had malaria.

Anemia was diagnosed in 56 (77%) participants with helminth infections, including all six with hookworm, all three with *S. haematobium*, the one with *A. lumbricoides,* and the one with *H. diminuta*. With regard to *Schistosoma* spp. infection (as determined by POC-CCA), 33 out of 46 infected had anemia (72%) and 12 of the 16 *S. stercoralis*-infected children were anemic (75%). Two children with anemia had both helminth infections and malaria.

Manuscript I

Table 5. Risk factors for Schistosoma and soil-transmitted helminth infections among preschool-aged children in Dar es Salaam, Tanzania.

Characteristics	All	*Schistosoma* spp.				Other helminths			
		Crude		Adjusted		Crude		Adjusted	
	n (%)	OR (95% CI)	p value	aOR (95% CI)	p value	OR (95% CI)	p value	aOR (95% CI)	p value
Age groups (months)			0.5		0.5		0.3		0.3
6-12	52(17)	1		1		1		1	
13-24	92(30)	1.33 (0.21-3.40)		1.31 (0.51-3.40)		2.36 (0.56-9.86)		2.38 (0.58-9.78)	
25-36	71(23)	0.89 (0.31-2.51)		0.86 (0.30-2.44)		2.00 (0.45-8.92)		1.91 (0.44-8.36)	
37-48	57(18)	0.76 (0.25-2.34)		0.76 (0.24-2.37)		0.80 (0.13-4.78)		0.78 (0.13-4.59)	
49-59	38(12)	1.00 (0.31-3.32)		0.92 (0.27-3.11)		0.80 (0.11-5.83)		0.80 (0.11-5.72)	
Sex			0.7		0.8		0.7		0.8
Female	160 (52)	1		1		1		1	
Male	150 (48)	1.12 (0.59-2.11)		1.06 (0.55-2.02)		1.16 (0.47-2.85)		1.05 (0.44-2.51)	
Individual deworming history [1]			0.9		0.9		0.6		0.6
Not dewormed	304 (98)	1		1		1		1	
Dewormed	6(2)	1.07 (0.11-10.12)		1.06 (0.11-10.1)		2.14 (0.17-26.84)		2.24 (0.18-27.20)	
TB exposure			0.3		0.4		0.5		0.6
Unexposed	121 (39)	1		1		1		1	
Exposed	189 (61)	1.43 (0.73-2.82)		1.34 (0.67-2.68)		0.72 (0.31-1.67)		0.74 (0.31-1.74)	
Number of people in the household			0.7		0.7		0.4		0.4
<6	190 (61)	1		1		1		1	
≥6	120 (39)	1.13 (0.59-2.16)		1.13 (0.58-2.20)		1.47 (0.61-3.53)		1.49 (0.62-3.54)	
Water source			0.2		0.3		0.3		0.3
Bore well	90(29)	1		1		1		1	
Tap	153 (49)	0.43 (0.21-0.88)		0.43 (0.20-0.94)		4.02 (1.04-15.5)		4.12 (1.05-16.3)	
Unknown	67(22)	0.41 (0.16-1.02)		0.41 (0.16-1.08)		4.22 (0.96-18.5)		4.90 (1.07-22.3)	
Type of toilet			0.04		0.04		0.9		0.9
Pit latrine	217 (70)	1		1		1		1	
Septic tank	93(30)	2.03 (1.03-4.00)		2.04 (1.02-4.07)		0.98 (0.37-2.57)		0.92 (0.35-2.40)	
Hygienic practices [2]			0.5		0.8		0.3		0.3
Poor	36(12)	1		1		1		1	
Better	274 (88)	0.74 (0.29-1.87)		0.87 (0.34-2.25)		0.55 (0.17-1.82)		0.54 (0.16-1.78)	
Household income per month (USD) [3]			0.5		0.3		0.3		0.4
<100	108 (35)	1		1		1		1	
≥100	202 (65)	1.25 (0.63-2.45)		1.56 (0.78-3.13)		0.61 (0.26-1.44)		0.71 (0.29-1.73)	
Parent education level			0.2		0.2		0.4		0.5
No or primary education	244 (79)	1		1		1		1	
Secondary/higher education	66(21)	0.57 (0.24-1.36)		0.53 (0.22-1.28)		0.60 (0.18-1.97)		0.63 (0.19-2.09)	

Parent occupation			0.9		0.7		0.4	0.5
Housewife/unemployed	196(63)	1		1		1	1	
Employed	114(37)	1.01 (0.52-1.96)		0.85 (0.42-1.76)		0.65 (0.25-1.66)	0.70 (0.26-1.92)	
Family migration history since			0.08		0.1		0.07	0.1
Migrated	23(7)	1		1		1	1	
Did not migrate	189(61)	1.58 (0.43-5.84)		1.55 (0.41-5.80)		4.74 (1.28-17.63)	5.30 (1.43-9.74)	
Unknown	98(32)	0.74 (0.18-3.09)		0.74 (0.18-3.10)		2.24 (0.89-5.63)	2.04 (0.80-5.18)	

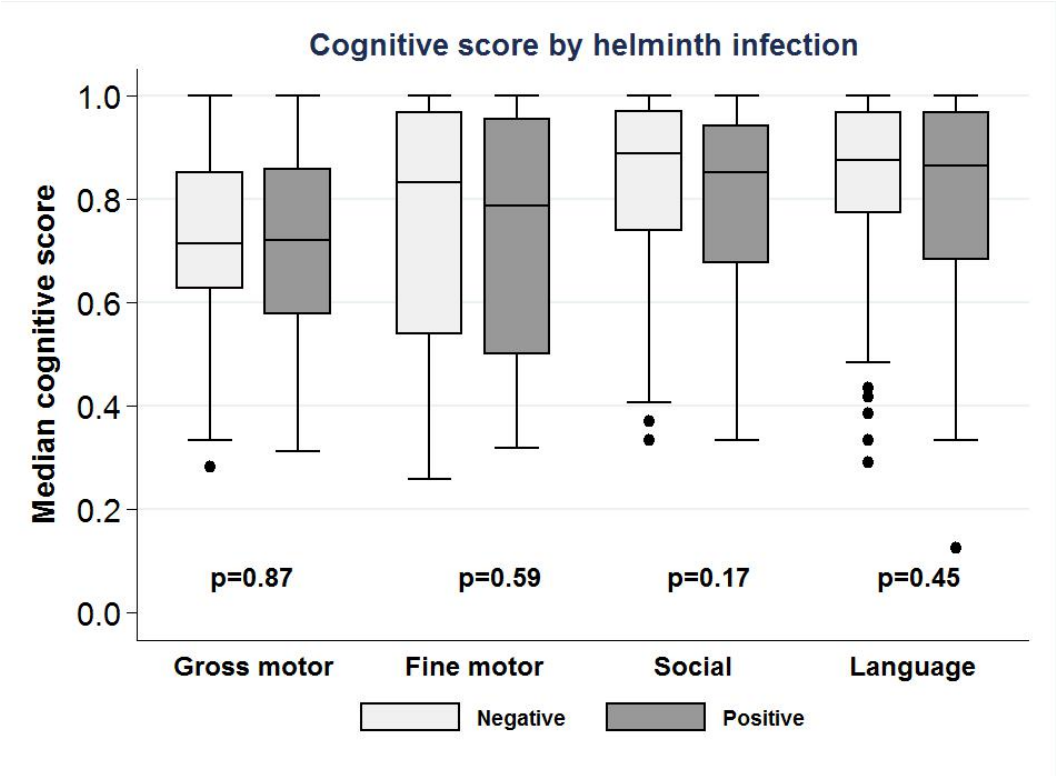
Schistosomiasis includes *S. mansoni* and *S. haematobium* (positive POC-CCA test results 1+, 2+, 3+ and egg-positive urine filtration); other helminth species (based on stool microscopy) include *A. lumbricoides*, *E. vermicularis*, hookworm, *H. diminuta*, and *S. stercoralis*

[1] Past three months

[2] Hygiene practice: parent/caregiver always wash fruits or vegetables before giving to children

[3] USD, United States dollars (1 USD=2,190 Tanzanian shillings)

Multivariable mixed logistic regression model with random intercepts at the level of matched pairs, containing the respective variable along with age, sex, type of toilet



Based on Swahili translation of MDAT score, Gladstone *et al*.,. The score has four components; gross motor, fine motor, social and language. P values are from logistic regression on a dichotomized median

Figure 10. Box plots comparing development and cognitive function among children with and without helminth infection.

When comparing hemoglobin and hematological parameters with helminth infection, the median hemoglobin value was significantly lower in helminth-infected children compared with their uninfected peers (10.1 g/dl [IQR 9.1±10.8 g/dl] vs. 10.4 g/dl [IQR 9.4±11.4 g/dl], p = 0.027) (Figure 11). This difference remained significant even when excluding malaria cases (10.4 g/dl [IQR 9.6±11.4 g/dl] vs. 10.1 g/dl [IQR 9.0±10.8], p = 0.014). All other hematological parameters (MCV, MCH, and RCDW) were equally distributed between helminth-infected and uninfected children.

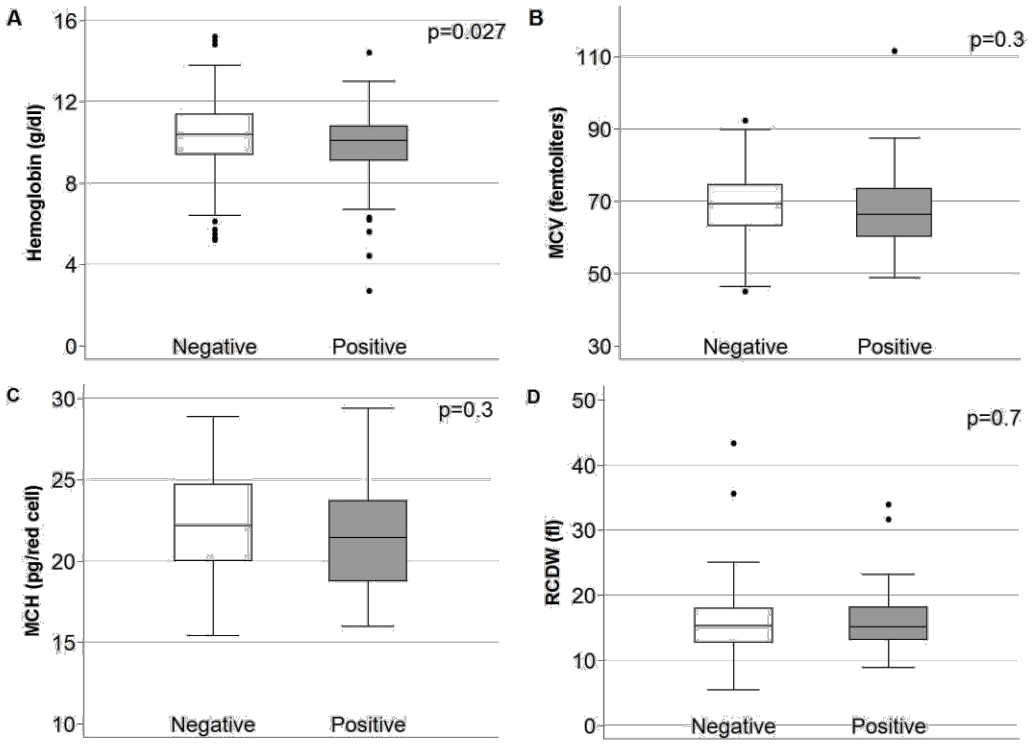


Figure 11. Box plots showing distribution of hemoglobin and red blood cell indices among children with (n=73) and without helminth infection (n=234).

A). Distribution of hemoglobin by helminth infection B). Distribution of mean corpuscular volume by helminth infection C). Distribution of mean corpuscular hemoglobin by helminth infection. D). Distribution of red blood cell distribution width by helminth infection.

2.5. Discussion

We present findings on the prevalence, clinical relevance, and risk factors associated with helminth infection among preschool-aged children in a poorly planned and under-resourced district in the coastal region of Dar es Salaam, Tanzania. We found that the prevalence of *Schistosoma* spp. was high (16.0%) among children under the age of 5 years, but the prevalence of other helminth infections was relatively low. We found no positive associations between helminth infections and commonly reported risk factors or development/cognitive scores. Anemia was a common clinical presentation and more frequent among children infected with helminths than their non-infected counterparts.

To our knowledge, this is the first study to report such a high prevalence of *Schistosoma* spp., as determined by the POC-CCA urine cassette test among preschool-aged children in the coastal urban area of Dar es Salaam. The POC-CCA is considered a highly sensitive rapid diagnostic test and was primarily developed for the detection of *S. mansoni.* In Tanzania, the POC-CCA has previously been used among preschool-aged children to detect *S. mansoni*, reporting a high prevalence of up to 50% in well-known high-risk *S. mansoni* areas around Lake Victoria (North-Western part of Tanzania), where the natural open freshwater serves as a habitat for the intermediate snail hosts (Mazigo et al., 2012, Ruganuza et al., 2015).However, a recent systematic review highlighted a low sensitivity of the POC-CCA test assay in detecting *S. mansoni* (as compared with stool microscopy), suggesting a higher sensitivity of the POC-CCA (as compared with stool microscopy) and/or the possibility of cross-reactivity of the assay with *S. haematobium* (Ochodo et al., 2015). In our study, the positive POC-CCA results were not confirmed by stool microscopy, since the commonly used Kato-Katz failed to identify any *S. mansoni* eggs in our study population. Furthermore, the urine filtration only revealed a very low prevalence of *S. haematobium* (1%). Similarly, in a recent investigation in Dar es Salaam, that used Kato-Katz and urine filtration, the prevalence of *S. haematobium* among school-aged children was reported to be 1.2%, while no *S. mansoni* was reported (Mwakitalu et al., 2014). Likely, the conventional stool and urine examination underestimate the true prevalence due to their low sensitivity to detect light intensity infection as they might occur in young children. However, an over-

estimation of *Schistosoma* spp. prevalence by a potential cross-reactivity of the POC-CCA with other conditions can also not be fully ruled out (Ochodo et al., 2015).

Urban schistosomiasis caused by *S. mansoni* has been reported elsewhere, including Brazil (Cabello et al., 2016), Côte d'Ivoire (Matthys et al., 2007) and Tanzania (Olsen et al., 2015), but most of these studies did not include preschool-aged children. However, intense transmission of *S. mansoni* has never been formally demonstrated in urban regions of Tanzania such as Dar es Salaam (Sarda et al., 1985, Brooker and Clements, 2009). Dar es Salaam is a coastal city along the Indian Ocean, was known to have a high prevalence and transmission of *S. haematobium* since the 1980s (Sarda et al., 1985, Mazigo et al., 2012). Our study showed that the prevalence of *S. haematobium* and *S. mansoni* infection as determined by egg counts in urine and stool is low, while the POC-CCA suggests that infections due to *Schistosoma* spp. have a considerably higher prevalence. Further studies with highly sensitive tests for *Schistosomiasis* (e.g. POC-CCA or polymerase chain reaction) in coastal Tanzania involving different age and population groups should be conducted to establish the species- and age-specific prevalence as the global focus is shifting towards disease elimination.

Overall, the prevalence of other helminth infections was found to be lower than that reported in other under-resourced settings (Yirgalem G/Hiwot et al., 2014, Alemu et al., 2016). Ten years ago, a study in two district hospitals in Dar es Salaam reported a soil-transmitted helminth prevalence of 33% among children <5 years (Kalison and Mwambete, 2006). The lower rates noted in our study may be due to an improved socioeconomic status among the general population and/or to successful biannual preventive chemotherapy campaigns, initiated in 2004, that include administering mebendazole and vitamin A supplementation to preschool-aged children (Clarke et al., 2017).

We did not find any association between helminth infections and commonly reported risk factors such as age, hygiene, low socioeconomic status, and history of migration. This is in contrast to other studies, which identified age, poor hygiene, and low socioeconomic status as risk factors for helminth infection in children (Davis et al., 2014, Ruganuza et al., 2015, Worrell et al., 2016, Alemu et al., 2016). The lack of association

with risk factors might be in part due to our sampling strategy, which was primarily powered to detect the prevalence of helminth infection among our study population, rather than association with risk factors. Although we identified having toilets with septic tanks as a risk factor for *Schistosoma* infection, this association lacked statistical significance after including POC-CCA trace results. We did not find evidence of an association between helminth infection and TB exposure. To our knowledge, no study has yet specifically investigated schistosomiasis and TB in preschool-aged children. However, a study in Kenya reported increased odds of hookworm infection among school-aged children with latent TB infection compared to unexposed controls (Sachiyo Nagi, 2013). It will be important to further elucidate the impact of helminth co-infections in early childhood on developing TB.

We documented a high prevalence of anemia among preschool-aged children that was associated with helminth infection. Similar findings have been reported in studies from Ethiopia and Nigeria, where children who were infected with two or more helminth species were at higher risk of having anemia (Yimam et al., 2016). High prevalence of anemia among preschool-aged children might also be caused by poor diets, low socioeconomic status of parents or caregivers, as indicated by the high rate of unemployment (National Bureau of Statistics and Regional Commissioner's Office, 2014, Ministry of Health Community Development Gender Elderly and Children et al., 2016). Other assessed hematological parameters were not associated with helminth infection, possibly due to low prevalence and intensity of helminth infection as well as to the good nutritional status among children evidenced by HAZ and WAZ in our study (Erismann et al., 2017). Previous research showed that heavy helminth infection impairs development and cognition (Jukes et al., 2002, Yentur Doni et al., 2015). In our study, helminth infection was not associated with reduced development and cognition. However, such differences may be seen only over-time during follow-up.

Our study has strengths and limitations that warrant further consideration. We systematically screened for helminthiases and other diseases, such as malaria, HIV, and active TB, using a suite of standardized and quality-controlled diagnostic tests (Mazigo et al., 2012, Mandalakas et al., 2012, Graham et al., 2012).

These infectious diseases all contribute to high morbidity and mortality among children <5 years (Albonico et al., 2008). The main limitations of our study include sampling households based on TB exposure (given that the overall study aim was to explore interactions of TB and helminth co-infections) and restricting the study area to an urban setting. However, poorly planned urban settings have the highest population growth in sub-Saharan Africa with considerable disease burdens of major infectious and non-communicable diseases (Zaman et al., 2015).

In conclusion, our study showed high prevalence of *Schistosoma* spp. infection as determined by the POC-CCA urine cassette test, among preschool-aged children, even in a highly urbanized setting in East Africa an observation that; has not been previously reported. It must be noted though that this result was achieved with a highly sensitive diagnostic assay, namely, the POC-CCA urine cassette test. Cross-reactivity with other conditions cannot be ruled out. Helminth infections were associated with anemia, but not with growth development and development of cognitive functions among our group of young children. However, the fact that helminth infection was not shown to affect children's development and cognition does not mean they will not be affected later in life. With the WHO's ambitious goal of reaching 100% coverage of preventive chemotherapy targeting major helminthiases, our findings call for urgent planning and implementation of specific interventions to prevent further morbidity, and to improve health, care, and wellbeing of these young children. Deworming likely reduces the prevalence of anemia, improves children's development and cognition, and prevents complications later in life (Yimam et al., 2016, Andrews et al., 2017). Future research to confirm our findings using newly developed and highly sensitive and specific test assays, identify and map *Schistosoma* spp. infection hotspots and its intermediate host snails in Dar es Salaam is needed to design targeted interventions for effectively controlling morbidity due to schistosomiasis and shift toward interruption of transmission.

Manuscript I

Acknowledgments

We thank all parents and caregivers who allowed their children to participate in this study. We are grateful to the Temeke Municipality for allowing us to conduct the study at Temeke hospital. We are also grateful to the Neglected Tropical Disease Program and TB coordinator in the municipal and the National TB and Leprosy Control Program for their support.

Supplementary Information

Supplementary Table 1. Risk factors for *S. mansoni* infection (defined trace results as positive based on Point of Care-Circulating Cathodic Antigen test) among 310 under-five children in Temeke District, Dar es Salaam, Tanzania.

Characteristics	All	Crude		Adjusted	
	n (%)	OR (95% CI)	p value	aOR (95% CI)	p value
Age groups (months)					
6-12	52 (17)	1	0.3	1	0.2
13-24	92 (30)	0.78 (0.35-1.72)		0.77 (0.35-1.70)	
25-36	71 (23)	0.76 (0.33-1.74)		0.74 (0.32-1.71)	
37-48	57 (18)	0.78 (0.32-1.88)		0.76 (0.31-1.84)	
49-59	38 (12)	0.50 (0.18-1.41)		0.47 (0.16-1.35)	
Sex					
Female	160 (52)	1	0.9	1	0.9
Male	150 (48)	0.97 (0.57-1.66)		0.97 (0.56-1.66)	
Individual deworming history (past 3)					
Not dewormed	304 (98)	1	0.9	-	-
Dewormed	6 (2)	1.10 (0.17-7.23)		-	
TB exposure					
Unexposed	121 (39)	1	0.8	-	-
Exposed	189 (61)	1.08 (0.64-1.84)		-	
Number of people in the household					
<6	190 (61)	1	0.7	-	-
≥6	120 (39)	1.12 (0.65-1.91)		-	
Water source					
Bore well	90 (29)	1	0.4	-	-
Tap	153 (49)	0.42 (0.23-0.75)		-	
Unknown	67 (22)	0.51 (0.25-1.05)			
Type of household toilet					
Pit latrine	217 (70)	1	0.4	1	0.4
Septic tank	93 (30)	1.25 (0.71-2.23)		1.30 (0.73-2.33)	
Hygienic practices[1]					
Poor	36 (12)	1	0.5	1	0.5
Better	274 (88)	0.76 (0.34-1.70)		0.77 (0.34-1.77)	
Household income per month (USD)					
<100	108 (35)	1	0.7	-	-
≥100	202 (65)	0.88 (0.50-1.55)		-	
Parent education level					
No or primary education	244 (79)	1	0.5	1	0.5
Secondary/higher education	66 (21)	0.80 (0.42-1.55)		0.81 (0.41-1.60)	
Parent occupation					
Housewife/unemployed	196 (63)	1	0.2	-	-
Employed	114 (37)	0.70 (0.40-1.20)		-	
Family migration history since child was born					
No	188 (61)	1	0.5	-	-
Yes	22 (7)	0.75 (0.26-2.16)		-	
Unknown	99 (32)	0.83 (0.47-1.48)		-	

[1] hygiene practices=how often parents wash fruits or vegetable before giving to children USD, United States Dollars (1USD=2,190 Tanzanian shillings)

Multivariable model only contained age, sex, type of toilet, hygiene, parent education

Manuscript I

Supplementary Table 2. Comparison of cognitive score among helminth-infected and non-infected preschool-aged children in Dar es Salaam, Tanzania.

Characteristic	All	Cognitive score median (IQR)		p value
	n (%)	Helminth infected	Helminth non-infected	
All	310 (100)	3.22 (2.74-3.44)	3.33 (2.80-3.50)	0.2
Age groups (months)				0.9
6-12	52 (17)	2.96 (2.70-3.38)	2.99 (2.54-3.39)	
13-24	92 (30)	2.92 (2.58-3.27)	2.92 (2.72-3.30)	
25-36	71 (23)	3.22 (2.79-3.44)	3.23 (2.43-3.49)	
37-48	57 (18)	3.41 (3.38-3.51)	3.50 (3.38-3.62)	
49-59	38 (12)	3.40 (3.32-3.48)	3.49 (3.40-3.66)	
Sex				0.6
Female	160 (52)	3.32 (2.85-3.45)	3.32 (2.82-3.50)	
Male	150 (48)	3.07 (2.61-3.38)	3.34 (2.77-3.50)	
Hemoglobin level (g/dl)				0.9
Anemic <11.0	203 (65)	3.08 (2.68-3.41)	3.21 (2.76-3.48)	
Not anemic ≥11.0	104 (34)	3.39 (3.04-3.48)	3.38 (2.98-3.57)	
Missing	3 (1)	-	-	
HAZ (z-score≤2)				0.9
Normal	243 (78)	3.33 (2.85-3.45)	3.36 (2.92-3.50)	
Moderate to severe stunted	67 (22)	2.85 (2.28-3.14)	2.91 (2.50-3.49)	
WAZ (z-score≤2)				0.9
Normal	228 (74)	3.31 (2.88-3.44)	3.32 (2.84-3.50)	
Moderate to severe underweight	82 (26)	2.91 (2.17-3.42)	3.40 (2.56-3.57)	
WHZ (z-score≤2)				0.7
Normal	225 (73)	3.07 (2.78-3.43)	3.32 (2.81-3.50)	
Moderate to severe wasted	79 (25)	3.33 (2.61-3.45)	3.42 (2.73-3.57)	
Overweight/Obese	6 (2)	2.98 (2.57-3.39)	2.95 (2.93-3.01)	
Deworming status (past 3 months)				0.9
Not dewormed	304 (98)	3.22 (2.70-3.43)	3.33 (2.81-3.50)	
Dewormed	6 (2)	3.32 (3.07-3.58)	3.09 (2.44-3.53)	
Household income per month (USD)				0.9
<100	108 (35)	3.11 (2.88-3.41)	3.23 (2.76-3.52)	
≥100	202 (65)	3.31 (2.68-3.45)	3.35 (2.82-3.50)	
Parent education level				0.9
No or primary education	244 (79)	3.22 (2.78-3.45)	3.34 (2.79-3.50)	
Secondary/higher education	66 (21)	3.06 (2.68-3.37)	3.30 (2.86-3.50)	
Parent occupation				0.9
Housewife/unemployed	196 (63)	3.07 (2.64-3.45)	3.24 (2.73-3.49)	
Employed	114 (37)	3.35 (3.01-3.43)	3.40 (2.91-3.55)	
Mothers HIV status during pregnancy				
Negative	256 (82)	3.28 (2.78-3.44)	3.34 (2.80-3.51)	-
Positive	30 (10)	2.88 (2.64-3.01)	3.26 (2.84-3.55)	
Unknown	24 (8)	1.99 (1.99-1.99)	3.37 (2.70-3.44)	
Mothers marital status				0.9
Single	76 (25)	2.96 (2.61-3.27)	3.22 (2.73-3.52)	
Married	217 (70)	3.33 (2.80-3.44)	3.35 (2.84-3.50)	
Unknown	17 (5)	3.29 (2.32-3.58)	3.12 (2.91-3.42)	

HAZ, height for age, moderate to severe stunting (z-score≤-2); WAZ, weight for age, moderate to severe underweight (z-score≤-2); WHZ, weight for height, moderate to severe wasting (z-score≤-2): HIV Human immunodeficiency virus

Manuscript II

3. Immunologic-based Diagnosis of Latent Tuberculosis among Children Less Than 5 Years of Age Exposed and Unexposed to Tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening

Khadija Said, MD MSc [1,2,3 ♠], Jerry Hella, MD MSc[1,2,3], Mwajabu Ruzegea, Ms. [1], Rajesh Solanki, MD MMed [4], Magreth Chiryamkubi, Mrs[5], Francis Mhimbira, MD PhD [1,2,3], Nicole Ritz, MD PhD[3,6], Christian Schindler, PhD [2,3], Anna M Mandalakas, MD PhD [7], Karim Manji, MD PhD [8], Marcel Tanner, PhD [2,3], Jürg Utzinger, PhD [2,3], Lukas Fenner, MD MSc [9 ♠]

1 Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania, **2** Swiss Tropical and Public Health Institute, Basel, Switzerland, **3** University of Basel, Basel, Switzerland, **4** Temeke Municipal Council Hospital, Dar es Salaam, Tanzania, **5** National Tuberculosis and Leprosy Control Program, Dar es Salaam, **6** Basel University Children's Hospital (UKBB), Basel, Switzerland, **7** The Global Tuberculosis Program, Texas Children's Hospital, Baylor College of Medicine, Houston, United States of America, **8** Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, **9** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

♠KS: +255 686 997 491,ksaid@ihi.or.tz and LF: +41 79 242 2066, lukas.fenner@ispm.unibe.ch

Conflicts of interest statement

Authors have none to disclose.

Funding

This work was supported by funding from the Rudolf Geigy Foundation (Basel, Switzerland). KS is a beneficiary of the Swiss Government Excellence Scholarship for Foreign Scholars and Artists (ESKAS).

Key words: Children under age of five years, Interferon-gamma release assay, Latent tuberculosis infection, Screening, Isoniazid Preventive Therapy.

Running head: TB screening in under-fives

Published Pediatric Infectious Disease Journal

Manuscript II

3.1. Abstract

Background: Childhood tuberculosis (TB) is acquired following exposure to an infectious TB case, often within the household. We prospectively screened children 6-59 months of age, exposed and unexposed to an infectious TB case within the same household, for latent tuberculosis infection (LTBI), in Dar es Salaam, Tanzania.

Methods: We collected medical data and clinical specimens (to evaluate for helminths, TB and HIV coinfections) and performed physical examinations at enrollment and at 3-month and 6-months follow-up surveys. LTBI was assessed using QuantiFERON (QFT) at enrollment and at 3 months.

Results: In total, 301 children had complete data records (186 with TB exposure and 115 without known TB exposure). The median age of children was 26 months (range 6-58); 52% were females, and 4 were HIV-positive. Eight children (3%) developed TB during the 6-month follow-up. We found equal proportions of children with LTBI among those with and without exposure: 20% (38/186) vs. 20% (23/115) QFT-positive, and 2% (4/186) vs. 4% (5/115) indeterminate QFT. QFT conversion rate was 7% (22 children) and reversion 8% (25 children). Of the TB-exposed children, 72% initiated isoniazid preventive therapy (IPT), but 61% of parents/caregivers of children with unknown TB exposure and positive QFT refused IPT.

Conclusions: In this high burden TB setting, TB exposure from sources other than the household was equally important as household exposure. Nearly one third of eligible children did not receive IPT. Evaluation for LTBI in children remains an important strategy for controlling TB, but should not be limited to children with documented TB exposure.

Manuscript II

3.2. Introduction

Tuberculosis (TB) continues to claim the lives of infected children younger than 5 years, and has been identified among the top 10 causes of death in this age group (Dodd et al., 2017). Young children typically acquire *Mycobacterium tuberculosis* infection from an infectious adult with pulmonary TB within the same household. Children with latent tuberculosis infection (LTBI) are at high risk of progressing to active TB disease within 12 months after infection; (Marais et al., 2004) and the risk is highest among children whose mothers are index cases (Beyers et al., 1997, Fox et al., 2013). Contact tracing and isoniazid preventive treatment (IPT) are recommended by the World Health Organization (WHO) to prevent active TB disease among high-risk populations, including under-5 year-old children. However, IPT uptake is a challenge; only 7% of children eligible for IPT received medication in 2015 worldwide (WHO, 2016a).

Despite the WHO's emphasis on providing TB preventive therapy, contact tracing remains a largely neglected part of TB control in high-burden countries due to lack of financial and human resources, coupled with the high costs associated with diagnostics and contact tracing itself (Oliveira et al., 2015). High-income countries mostly use interferon-gamma release assay (IGRA) for TB contact tracing, which has high specificity for detecting *M. tuberculosis* infection but limited sensitivity, particularly in children (Ritz and Curtis, 2014). Most TB programs in low-income countries and high TB-incidence rates use tuberculin skin test (TST) instead of IGRA, such as the ELISpot-based test (T-SPOT.TB) and the enzyme-linked immunosorbent assay (ELISA)-based test QuantiFERON-TB Gold (QFT), that are restricted to research use only in these settings (Shah et al., 2011, Rose et al., 2012, Mandalakas et al., 2015). Due to the limitations of IGRAs for the diagnosis of LTBI in children in high TB incidence countries, a quantified exposure score was recently proposed to serve as a surrogate measure of *M. tuberculosis* infection, specifically in TB-exposed children (Mandalakas et al., 2012, Mandalakas et al., 2015).

In Tanzania, the National TB and Leprosy Control Program (NTLP) reported that childhood TB cases constituted 10% of the country's TB cases in 2004–2012 (NTLP and MoHSW, 2012). To interrupt transmission of TB and combat the childhood TB epidemic in the country, since 2013, the program has

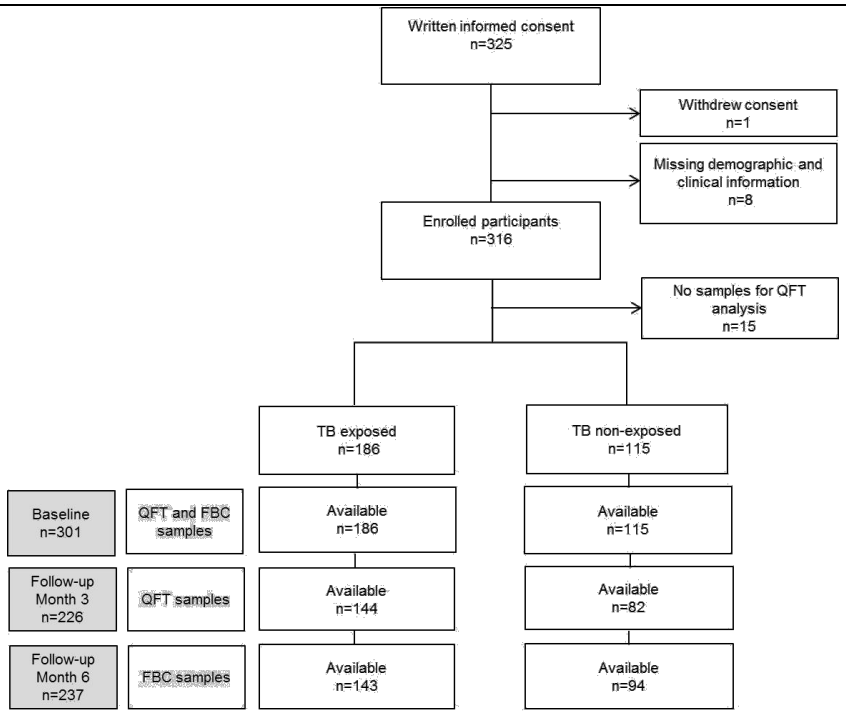
been working to improve childhood TB case detection and reporting. However, the yield of infection screening, particularly among children without documented TB exposure, and the implications for screening strategies in routine settings has not yet been investigated (Lobato et al., 2000). We conducted a prospective study among children under the age of 5 years exposed to infectious pulmonary TB cases in their households and controls from neighboring households, to assess the prevalence of LTBI among under-5 year-old children in a high TB burden setting in Tanzania, using immunodiagnostic tests and a quantified TB exposure score.

3.3. Material and Methods

Study Setting and Design

Temeke is one of three districts in Dar es Salaam, the economic capital of Tanzania, with an estimated 1.4 million residents. The under-five mortality in Dar es Salaam is 72 per 100,000 (National Bureau of Statistics and Office of Chief Government Statistician, 2015). In 2014, TB prevalence in adults was 270 per 100,000 population in Temeke (NTLP and MoHSW, 2015). HIV prevalence among pregnant mothers in the district is estimated to be 6% (National Bureau of Statistics and Regional Commissioner's Office, 2014). The NTLP conducts routine contact tracing of young children by counselling smear-positive adult TB cases to bring children under the age of 5 years from their households for symptom-based TB screening and IPT if not found to have disease.

We conducted a prospective study in Temeke district among TB-exposed children, with the overarching aim of exploring TB/helminth co-infection, as previously described (Said et al., 2017a). To recruit controls, we invited children from neighboring households according to standard operating procedures (Supplemental Digital Content 1, standard operating procedure), (Grimes and Schulz, 2005) and after asking if there was an adult on TB treatment or suspected of having TB in the household. Children were referred to the Reproductive and Child Health (RCH) Clinic at Temeke Municipal Hospital (where children under the age of 5 years are typically seen) for enrollment and follow-up visits at 3 and 6 months after enrollment (Figure 12).



FBC; Full Blood count; QFT, QuantiFERON-TB Gold; TB, Tuberculosis

Figure 12. Selection of study participants and blood samples.

Study Population and Sample Size Consideration

We invited 398 parents/caregivers of children aged 6–59 months, between October 2015 and September 2016. Children were included if: (i) their parents/caregiver provided written informed consent; (ii) they were aged 6–59 months at the time of enrollment; (iii) they resided within Temeke district and would continue to do so in the next 6 months after enrollment; and (iv) they lived with a smear-positive adult pulmonary TB case, as diagnosed by sputum smear microscopy and culture (Mhimbira et al., 2017) (for case children) or came from households without documented TB case (for control children) (Appendix 1: Standard Operating Procedure: Invitation of control children). Children with known TB exposure were recruited within a month of the index case starting TB treatment. We aimed for a sample size of 300 children aged 6–59 months, with 150 TB-exposed and 150 TB-unexposed children. This sample size was estimated to provide a precision of 5% in estimating TB infection prevalence, with an error probability of 5%.

Study Procedures

***At Enrollment**:* The study clinician collected demographic and socioeconomic information and, using a standardized case report form (CRF), evaluated children for the following TB signs and symptoms: (i) current cough of any duration; (ii) abnormal fatigue or reduced playfulness; (iii) documented weight loss or failure to thrive in the past 3 months; (iv) axillary temperature >37.5 °C; (v) visible cervical mass (>2 x 2 cm); (vi) gibbus (sharp angular spine deformity); and (vii) other signs suggestive of possible extra-pulmonary TB. The clinician also collected histories on feeding practices, prior illnesses (including TB), previous use of IPT and other medications, and recorded vaccination history (including BCG vaccination), which was verified on the RCH card. All children had chest X-rays taken. Chest X-rays were interpreted by two independent radiologists; in case of discrepancies, a third radiologist was asked to resolve the discrepancies. Trained study nurses recorded anthropometric measurements, including height and weight, and collected venous blood for quantiFERON (QFT), full blood counts (FBC), HIV testing, and malaria screening (see also Laboratory Procedures below).

The nurses also performed sputum induction the morning after enrollment and collected induced sputum samples for Xpert MTB/RIF (Cepheid; Sunnyvale, CA, USA) (Zar et al., 2005, Sabi et al., 2016).

Parents/caregivers were given two pre-labeled containers and were instructed to bring urine and stool samples from their children the following day for screening of helminth infection. Additionally, each participant was provided with a plastic pocket that had an adhesive tape (50 x 20 mm) and a pre-labeled glass slide and asked to submit the slide with anal adhesive tape for *Enterobius vermicularis* examination (Salim et al., 2014).

***At 3-Month and 6-Month Follow-Up Visits**:* Children were clinically evaluated for TB and other medical conditions. We collected blood samples for QFT analysis at the 3-month visit and for FBC at the 6-month visit. Clinical management was determined based on case presentation.

***TB Exposure Score*:** We used a TB exposure score chart, adopted from South Africa, to assess TB exposure (Mandalakas et al., 2012). The chart consists of 10 questions: (i) is the child's mother the index case?; (ii)

is the child's primary caregiver the index case?; (iii) does the child sleep on the same bed as the index case?; (iv) does the child sleep in the same room as the index case?; (v) is the index case coughing?; (vi) is the index case a pulmonary TB case?; (vii) does the index case have smear positive TB?; (viii) does the index case live in the household with the child?; (ix) does the index case see the child every day?; and (x) is there more than one TB case in the household? Control participants were not assessed for TB exposure.

***Screening for, and Diagnosing Active TB Disease*:** We used a comprehensive symptom-based TB tool to screen for active TB disease at each visit (Appendix 2: Tuberculosis screening tool). At baseline, in addition to symptom-based screening, we performed chest X-rays and Xpert MTB/RIF for every child within 3 days after enrollment (see also Laboratory procedures below). At follow-up visits, we used the symptom-based screening tool; X-ray and/or Xpert MTB/RIF were ordered only if there was an indication. Confirmation and final diagnosis were based on clinical evaluation, chest X-ray findings and Xpert MTB/RIF, according to Graham et al (Cuevas et al., 2012)**.**

Children known to have TB exposure but without active disease were started on IPT (20 mg/kg), in accordance with the Tanzanian NTLP treatment guideline (NTLP and MoHSW, 2012). Parents/caregivers of children with positive QFT results but without documentation of exposure to an infectious TB case were counselled and advised to start their children on IPT. Children diagnosed with TB were referred to NTLP for TB treatment. All children diagnosed with medical conditions were clinically managed in accordance with Tanzanian national guidelines.

Laboratory Procedures

Microbiologic investigations*:* We performed Xpert MTB/RIF (Cepheid; Sunnyvale, CA, USA) on the induced sputum samples (Sabi et al., 2016) to diagnose active TB disease in the laboratory at Temeke district hospital. The laboratory is subject to continuous quality control monitoring by the Central Tuberculosis Reference Laboratory (Dar es Salaam, Tanzania).

QuantiFERON -TB Gold (QFT): We used venous blood to measure *M. tuberculosis*-specific T-cell responses, measuring levels of interferon-gamma released in whole blood in response to stimulation with

the *M. tuberculosis*-specific antigens. The blood was collected in three QFT tubes (QuantiFERON-TB Gold, Cellestis; Carnegie, Victoria, Australia): TB-Ag (coated with MTB specific antigens), Mitogen (positive control coated with phytohaemagglutin), and Nil (negative control coated with saline). The blood was transferred to the laboratory within 1 hour and incubated at 37°C for 16–18 hours. After incubation, the samples were centrifuged and supernatants stored at -80° C pending further processing by ELISA to detect interferon-γ (IFN-γ) response associated with *M. tuberculosis* infection. Results were interpreted according to the manufacturer's instructions.

Other blood tests: HIV screening was done with Alere Determine HIV-1/2 (Alere; Holliston, MA, USA) among children aged ≥18 months, or PCR for children aged <18 months. Full blood cell counts were done using a MS4 Vet hematology analyzer (Diamond Diagnostics; Holliston, MA, USA).

***Helminth investigations*:** Stool and urine samples were examined for helminth infections using the Kato-Katz, FLOTAC, Baermann, direct microscopy methods and a point-of-care circulating cathodic antigen (POC-CCA) urine cassette test for *Schistosoma mansoni* diagnosis, as previously described (Said et al., 2017a, Salim et al., 2014).

Definitions

We recorded clinical data directly onto tablet computers using the open-source software, open data kit (ODK; http://opendatakit.org/), and the data management tool, odk_planner (Steiner et al., 2016). The TB exposure score was categorized into not likely to have TB infection (score of 1–6) or presumptive TB infection (score of ≥7). A composite QFT result was defined as positive QFT at either baseline or 3-month follow-up. A child was considered TB infected if the QFT result was positive. A child was considered to have active TB (confirmed or probable) according to international consensus from an Expert panel (Graham et al., 2012). Chest X-ray features suggestive of TB included consolidation, cavities and hilar lymphadenopathy. Children diagnosed with active TB disease three months after enrollment were defined as coprevalent TB cases, while children diagnosed with TB three months after enrollment were defined as incident TB cases (Mandalakas et al., 2015). Anemia was defined as hemoglobin (Hb) <11.0g/dl, as per

WHO recommendations, further stratified into mild (Hb: 10-10.9 g/dl), moderate (Hb: 7.0-9.9 g/dl), and severe anemia (Hb: <7.0 g/dl) (WHO, 2011a). Anthropometric z-scores were calculated using the 2006 WHO Growth Standards in Stata version 13.1 (Stata Corp. LLC; College Station, TX, USA), using the zscore06 command (WHO, 2006). Helminth infection was defined as infection with any of the following helminth species: *Ascaris lumbricoides*, *E. vermicularis*, *Hymenolepis diminuta*, hookworm, *Schistosoma haematobium*, *S. mansoni*, *Strongyloides stercoralis*, and *Trichuris trichiura*.

Statistical Analysis

Frequencies and proportions were used to describe the children's demographic and clinical characteristics, both overall and stratified according to the presence or absence of documented TB exposure. QFT indeterminate results were excluded in the LTBI risk factor analysis. We performed mixed logistic regression analyses to identify risk factors for LTBI taking into account the matching of TB-exposed and unexposed children. These analyses were conducted with and without interaction terms between the respective risk factor variables and TB-exposure status. Additionally, we ran stratified analyses separating TB-exposed and unexposed children.

We generated an exposure scale based on the TB exposure score (0, no exposure; 1–4, minimal exposure; 5–6, medium exposure; and 7–10, maximum exposure). We constructed a core multivariable mixed logistic regression model for QFT-conversion and reversion between baseline and follow-up, comprising age, sex, and lymphocyte count, and alternatively added TB exposure, IPT during follow-up, and helminth infection as potential risk factors for QFT conversion and reversion. For children documented to have TB exposure, we also added TB exposure score and mother being index case as potential risk factors for QFT conversion and reversion. We also presented graphically the paired QFT results at enrolment and after 3 months, separately for QFT converters and reverters to illustrate the distribution of IFN-γ values during the two visits for children who converted to QFT positive, reverted to QFT negative, and those who did not change. All analyses were performed in Stata version 13.1 (Stata Corporation; College Station, TX, USA).

Manuscript II

Ethics Statement

The study was approved by the Institutional Review Board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/No: 12-2015), the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (NIMR; reference no. NIMR/HQ/R.8a/Vol. IX/2002) and by the Ethics Committee of the North-west and Central Switzerland (EKNZ reference no. UBE-15/49). All children were recruited only after their parents/caregivers gave written informed consent.

Manuscript II

3.4. Results

Children baseline characteristics

Of the 398 children aged 6-59 months invited to participate in the study, 325 parents/caregivers provided written informed consent, and hence, their children were enrolled. Clinical, demographic, and socioeconomic information were obtained from 316 children. Fifteen children did not provide blood samples for QFT analysis; thus, 301 children were included in the final analysis (Figure 12).

Among the 301 child participants, 186 (62%) were exposed to adults with smear-positive pulmonary TB, and 115 (38%) were not known to have been exposed to TB. Overall, the median age was 26 months (range 6-58 months); 156 (52%) were girls and four were HIV-positive (Table 6). All children were BCG vaccinated based on vaccination documents. TB-exposed children were, on average, older than children without known TB exposure. Ninety-five (32%) children presented with signs and symptoms suggestive of TB (Supplementary. Figure 1).

A total of eight (2.7%) children developed active TB during follow-up (six in the exposed, two in the unexposed group). All TB cases were diagnosed based on a clinical case definition, (Graham et al., 2012) which included patient's history, chest radiography, positive response to anti-TB medication, and evidence of TB infection. Five children (1.7%) were considered co-prevalent TB cases (three exposed to infectious TB cases); four of them were <2 years, four were boys, and one was HIV-positive.

Three children were considered incident TB cases; one was <2 years, and none was HIV positive. The proportion of children with active TB (prevalent and incident) in the exposed and non-exposed group was statistically not significantly different (3.2% *vs.* 1.7%, p=0.44). Children exposed to HIV-seropositive mothers were not more likely to have active TB compared to HIV non-exposed children (7% *vs.* 2%, p=0.15). All children with TB had mild or moderate anemia.

Table 6. Baseline demographic, socio-economic and clinical characteristics of 301 under-five children, enrolled October 2015-Septeber 2016, and their parents/caregivers in Dar es Salaam, Tanzania

Characteristic n (%)	TB exposed n=186	TB unexposed n=115
Child characteristics		
Age (months), median (IQR)	27 (18-43)	25 (14-37)
Age groups (months)		
6-23	73 (39)	56 (49)
24-59	113 (61)	59 (51)
Sex		
Male	82 (44)	63 (55)
Female	104 (56)	52 (45)
BCG diameter (mm)		
No scar	40 (21)	10 (9)
Diameter 1-4	89 (48)	64 (56)
Diameter >4	57 (31)	41 (35)
HIV infection		
Positive	2 (1)	2 (2)
Hemoglobin level (g/dl)		
Anemic <11.0	119 (64)	76 (66)
Helminth infection		
Positive	44 (24)	26 (23)
TB exposure score [1]		
Likely infected	110 (59)	-
HAZ		
Median (IQR)	-1.16 (-1.92 to -0.31)	-1.00 (-1.90 to -0.18)
WAZ		
Median (IQR)	-1.28 (-2.19 to -0.42)	-0.81 (-1.80 to -0.20)
WHZ		
Median (IQR)	-1.01 (-2.11-0.13)	-0.65 (-1.53-0.16)
Signs and symptoms		
Fever	17 (9)	2 (2)
Any Cough	60 (32)	30 (26)
Failure to thrive	5 (3)	2 (2)

[1] Applicable to TB exposed study participants only

Current cough of any duration; Failure to thrive in the past three months; Fever: axillary temperature >37.5ºC; HAZ, height for age, moderate to severe stunting (z-score≤-2); HIV, Human immunodeficiency virus; IPT Isoniazid Preventive Therapy; TB exposure score (Mandalakas et al., 2012); WAZ, weight for age, moderate to severe underweight (z-score≤-2); WHZ, weight for height, moderate to severe wasting (z-score≤-2)

Prevalence of LTBI in TB Exposed and Unexposed Children

At enrollment, 15% (27/186) of TB exposed children were QFT-positive compared to 10% (12/115) of children without documented TB exposure (p=0.37). Indeterminate QFT results were also equally distributed between the two groups (9/115 [8%] vs. 9/186 [5%], p=0.37). At the 3-month follow-up visit

(composite results), QFT was positive in 20% (61/301) children: 20% (38/186) of TB exposed vs. 20% (23/115) of TB unexposed (p=0.9), as shown in Table 7. The prevalence of indeterminate results was 3% (9/301).

Of those children diagnosed with active TB during follow-up, 10% (4/39) had a positive QFT result at enrolment. Among co-prevalent TB cases, 2/5 had positive QFT results, and 2/3 incident TB cases were QFT positive.

A fitted model, taking clustering into account, did not suggest any correlation between QFT-positive results (indicating LTBI) and the TB exposure score (odds ratio (OR) 1.06, 95% confidence interval (CI) 0.81-1.40, p=0.7). There was also no correlation between LTBI and the presence of a BCG scar; all children had a documented BCG vaccination.

Risk Factors for LTBI

We found that TB-exposed children from households with up to five members had a higher risk of having LTBI (defined as a positive QFT test result) compared to all other children (OR 3.57, 95% CI 1.54-7.69, p=0.003) (Table 8). All other risk factors such as age, sex, lymphocyte count, having a mother with TB, sleeping in the same bed as the index case, and household income showed no association. Among children without documented TB exposure, none of the risk factors showed significant association with LTBI.

QFT Conversion, Reversion and Associated Patient Factors

Forty-seven (16%) children had different QFT results when retested at the 3-month visit: 22 (8%) children converted to QFT-positive at the 3-month follow-up, 11 (50%) of which had documented TB exposure. Twenty-five children (8%) reverted to negative QFT results at the 3-month follow-up. Considering variables such as sex, age category, average BCG scar size, lymphocyte counts, an index mother, isoniazid uptake, and infection with any helminth, we found none of the factors to be predictive for either QFT conversion or reversion (Supplementary. Table 3). Neither were coprevalent TB cases and incident cases associated with QFT conversion or reversion, Figure 13 shows the paired semi-quantitative QFT results at enrollment and at the three months follow-up visit among children who converted to QFT positive (Panel

A), Reverted to QFT negative (Panel B) and did not have any change of their QFT results (Panel C) at three month follow-up visit.

IPT Uptake Among exposed and unexposed children

By the six-month visit (end of follow-up), 72% (133/186) children exposed to an infectious TB case had started IPT (Table 2), and 28% (53/186) did not start IPT. Among the 53 who did not start IPT, 57% (30/53) parents/caregivers refused IPT, and 43% (21/53) were lost to follow-up. Among the 133 children who started IPT, 3% (4/133) developed active TB later on (two co-prevalent, two incident cases) and were started on anti-TB medications. Of the 53 exposed children who were not started on IPT, 6% (2/53) developed TB disease and started anti-TB medications (1 co-prevalent, 1 incident case).

Among 115 children with undocumented TB exposure, 20% (23/115) had an LTBI diagnosis based on QFT testing. Of these 23 children, one child started IPT, and one child developed TB later on (coprevalent case) and was started anti-TB medication; 61% (14/23) of parents/caregivers refused IPT; and 30% (7/23) were lost to follow-up.

Manuscript II

Table 7. Prevalence of latent tuberculosis infection (LTBI) based on composite quantiFERON-TB Gold (QFT) results among children under-five years in Dar es Salaam, Tanzania.

Characteristics	TB exposed (n=186)			TB unexposed (n=115)		
	QFT result			QFT result		
	Positive	Negative	Indeterminate	Positive	Negative	Indeterminate
Total n (%)	38 (20)	144 (78)	4 (2)	23(20)	87 (76)	5 (4)
Age (months), median (IQR)	33 (22-43)	26 (17-44)	29 (13-47)	26 (17-43)	24 (12-37)	20 (18-34)
Age groups (months)						
6-23	12 (32)	59 (41)	2 (50)	10 (43)	43 (491)	3 (60)
24-59	26 (68)	85 (59)	2 (50)	13 (57)	44 (51)	2 (40)
Female	26 (68)	77 (53)	1 (25)	12 (52)	38 (44)	2 (40)
BCG scar diameter (mm)						
No scar	9 (24)	29 (20)	2 (50)	1 (4)	9 (10)	0
1-4	16 (42)	71 (49)	2 (50)	14 (61)	46 (53)	4 (80)
>4	13 (34)	44 (31)	0	8 (35)	32 (37)	1 (20)
HIV positive	0	2 (1.4)	0	1(4)	1 (1.2)	0
>3people in the sleeping room	10 (26)	31 (22)	2 (50)	5 (22)	20 (23)	1 (20)
Mother index [1]	15 (39)	39 (27)	0			
Index other primary caregiver [1]	9 (24)	33 (23)	3 (75)	-	-	-
Sleep same bed as index [1]	28 (74)	87 (60)	3 (75)	-	-	-
Multiple indexes in h/hold [1]	5 (13)	13 (9)	0	-	-	-
Likely infected with TB [1]	25 (66)	82 (57)	3 (75)	-	-	-
Coprevalent TB cases [2]	1 (3)	2 (1.4)	0	1(4)	1 (1.2)	0
Incident TB cases [3]	2 (5)	1 (0.7)	0	0	0	0
IPT during follow-up	29 (76)	101 (70)	3 (75)	1(4)	0	0
Any helminth infection	10 (26)	32 (22)	2 (50)	8 (35)	17 (20)	1 (20)

LTBI defined as positive QuantiFERON-TB-Gold at either baseline or 3-month follow-up (composite QFT result). Children who developed TB during follow-up are included in this analysis. IPT, isoniazid prophylactic therapy; QFT, quantiFERON; LTBI, latent tuberculosis infection; TB, tuberculosis

[1] Applicable to TB-exposed group only based on TB exposure score (Mandalakas et al., 2012)

[2] Diagnosed within 3 months of enrollment

[3] Diagnosed after 3 months post enrollment

Manuscript II

Table 8. Risk factors for LTBI among children under-five year of age in Dar es Salaam, Tanzania.

Characteristics	All	TB exposed (n=182)		TB unexposed (n=110)	
n (%)	292	OR (95% CI)	p value	OR (95% CI)	p value
Age groups (months)			0.29		0.61
6-23	124 (42)	1		1	
24-59	168 (58)	1.50 (0.70-3.22)		1.27 (0.51-3.21)	
Sex			0.10		0.47
Male	139 (48)	1		1	
Female	153 (52)	1.89 (0.88-4.02)		1.41 (0.56-3.52)	
BCG scar diameter (mm)			0.63		0.39
No scar	48 (16)	1		1	
1-10	244 (84)	0.81 (0.34-1.90)		2.54 (0.30-21.13)	
HAZ			0.36		0.49
Normal	231 (79)	1		1	
Stunted	61 (21)	1.48 (0.64-3.40)		1.45 (0.50-4.24)	
TB clinical sign and symptoms			0.22		0.11
None	143 (49)	1		1	
At least one	149 (51)	1.58 (0.76-3.26)		0.45 (0.17-1.18)	
Hemoglobin level (g/dl)			0.17		0.48
Not anemic ≥11.5	99 (34)	1		1	
Anemic <11.5	189 (65)	0.60 (0.29-1.24)		0.71 (0.27-1.84)	
Lymphocyte count[1]			0.33		0.24
Normal	226 (77)	1		1	
Abnormal	66 (23)	0.60 (0.22-1.67)		1.79 (0.68-4.69)	
Any helminth infection			0.59		0.13
Negative	225 (77)	1		1	
Positive	67 (23)	1.25 (0.55-2.84)		2.20 (0.80-6.02)	
Mother index case[2]			0.14		-
No	238 (82)	1		-	
Yes	54 (18)	1.76 (0.83-3.71)		-	
Sleep same bed as index case[2]			0.13		-
No	177 (61)	1		-	
Yes	115 (39)	1.83 (0.83-4.06)		-	
Household income per month (USD)			0.36		0.19
<100	105 (36)	1		1	
≥100	187 (64)	1.41 (0.67-2.98)		0.53 (0.20-1.38)	

Household size			0.003	0.25
>5 people	121 (40)	1	1	
1-5 person	173 (60)	3.57 (1.54-7.69)	2.86 (0.64-5.6)	

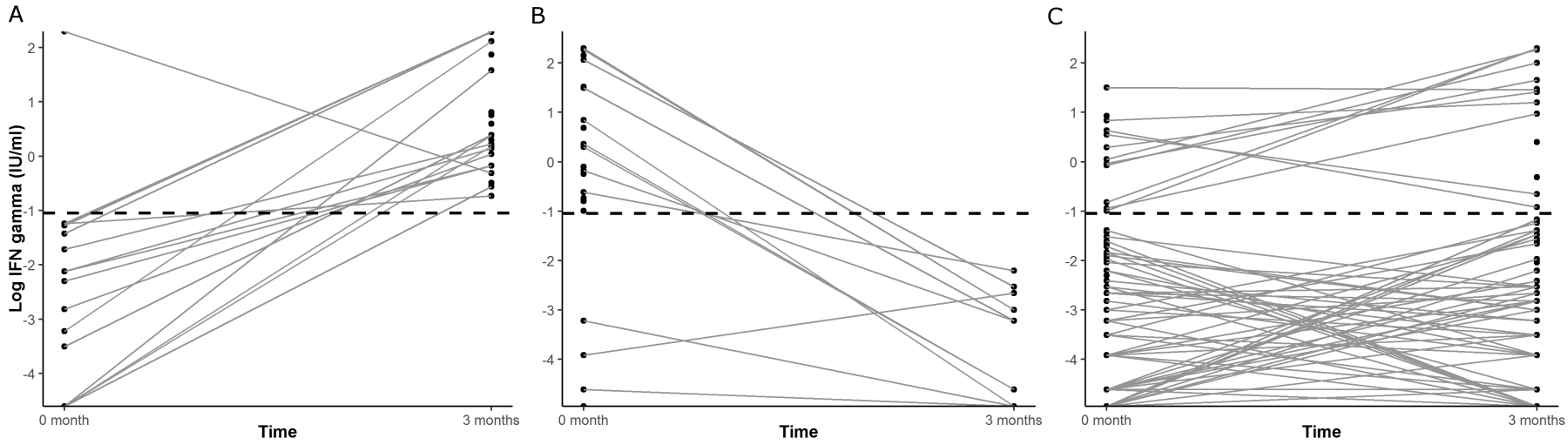
Nine children with indeterminate QFT results were excluded in this analysis. LTBI was defined as a positive QuantiFERON-TB-Gold (QFT) test at either baseline or 3-month follow-up.

H/hold, household; HIV, human immunodeficiency virus; USD, United States dollars (1 USD=2,190 Tanzanian shillings)

Estimates derived from bivariate mixed logistic regression models.

[1] Lymphocytes, normal 2.1-9.5, abnormal <2.1 or >9.5 $10^3/\mu l$

[2] Applicable to TB-exposed group only based on TB exposure score (Mandalakas et al., 2012)



Dashed lines represent cut off -1.05

Figure 13. Trends in the distribution of semi-quantitative quantiFERON (QFT) results IFN- values) among 226 children aged 6-59 months at recruitment and at the 3-month follow-up visit in Temeke district, Dar es Salaam, Tanzania between October 2015 and September 2016.

3.5. Discussion

We present findings from a prospective study of 186 TB-exposed children aged 6-59 months and 115 unexposed controls, who were followed-up for 6 months in the Temeke district in Tanzania's economic capital of Dar es Salaam, characterized by high TB notification rate. QFT diagnosed equal proportions of children with LTBI in the two groups. IPT uptake was low among TB-exposed children, and even lower among children with undocumented TB exposure.

We showed that, overall, TB infection among exposed under-5 year-old children was low, but interestingly similar proportions of children in the two groups (TB-exposed and unexposed) were diagnosed with LTBI. The positive QFT results among children that did not have known exposure to TB is likely the result of community exposure to infectious TB cases. Previous studies have suggested that young children are more likely to be infected in domiciliary settings, (Chabala et al., 2017, Seddon and Shingadia, 2014, Wood et al., 2010) but due to the high prevalence of the disease in our setting, community exposure cannot be ruled out. Our findings show a higher proportion of LTBI among TB-unexposed children (20%) compared to other studies that assessed LTBI by QFT in under-5 year-old children (range 2.2-17.9%) (Marquez et al., 2016, Bianchi et al., 2009, Masoumi Asl et al., 2015). Previously documented reasons associated with LTBI among children include visiting other households and poor ventilation in the visited households (Wood et al., 2010). However, our reported proportion of LTBI was considerably lower compared to other studies that report LTBI prevalence as high as 49% (Mandalakas et al., 2015). The lower LTBI prevalence in our study population may be due to a timely TB diagnosis in index cases, resulting in lower risk of transmission. Children from larger households were less likely to have QFT-positive results, indicating lower transmission resulting from less exposure time to an infectious TB case (Wood et al., 2010).

Our results suggest that IPT implementation in our study population remains a challenge. Indeed, about a third of the children eligible for IPT had not yet started treatment, as recommended by WHO in its roadmap for childhood TB (WHO, 2013).

It should be noted, however that a study in India reported a much lower uptake (22%), while more than half of the exposed children in our study were on medication (Singh et al., 2017). High-income settings use IGRA for TB screening, while many under-resourced settings use symptomatic screening techniques to initiate TB preventive therapy, due to high cost of IGRA tests (Mandalakas et al., 2015, Shah et al., 2011). The WHO recommends IPT in all children below the age of 5 years who are in contact with an infectious TB case or with proven TB infection after excluding active disease (WHO, 2013, WHO, 2014). IGRAs could be a useful tool to guide delivery of IPT in TB high burden settings, but screening strategy must consider transmission dynamics and population characteristic unique to each setting. This policy-practice gap that exists in high-burden settings needs to be addressed provided resources allow.

Contrary to other studies, our findings show a small proportion of children with indeterminate QFT results. The prevalence of indeterminate QFT results varies, especially among children (Perez-Porcuna et al., 2014, Thomas et al., 2010, Powell, 2009, Haustein et al., 2009, Bianchi et al., 2009). For instance, Italy reported a prevalence of 0.6%, while in South Africa, a prevalence of 2.5% was found. Compared to those two countries, our prevalence was considerably higher (Mandalakas et al., 2015, Bianchi et al., 2009). Malnutrition, helminth infection, and HIV infection are reportedly associated with indeterminate QFT results (Thomas et al., 2010, Haustein et al., 2009). However, in our study population, the proportion of indeterminate results was low even after repeating the test at the 3-month follow-up visit and considering composite QFT results, which we used to define the final results. Contrary to other studies, we did not find any association between indeterminate QFT results and helminths (Thomas et al., 2010).

Overall, a low proportion of children had active TB disease at the end-of-follow-up visit. Despite the high risk of disease progression in young children after TB infection, the number of children developing active TB disease in our cohort was very low compared to those in Italy, where 5% of children developed active TB disease (Bianchi et al., 2009). The majority of children diagnosed with active TB did not have radiological findings consistent with active TB disease.

This might be explained by a mild form of the disease diagnosed during presentation. However, in children, absence of radiologic findings can be explained by transient radiologic changes and the natural history of primary TB complex, which self-cures without intervention (Marais et al., 2004). Due to the low number of incident cases, we did not find sufficient evidence for risk factors associated with active disease. Despite systematic and thorough screening of active TB disease, none of the TB cases, neither coprevalent nor incident TB cases, were microbacteriologically confirmed with Xpert MTB/RIF. Although this may be due to the paucibacillary nature of childhood TB, this finding may also reflect early diagnosis of the disease at presentation in our study.

Our study has strengths and limitations to be considered. We encountered a few challenges, mainly during enrollment, that are worth highlighting. Several parents/caregivers, especially those whose children had no history of TB exposure, were reluctant to subject their children for screening and were concerned with the sputum induction procedure despite intensive counseling. All children were systematically screened for TB, HIV, malaria, and helminth infection, as all of these diseases contribute to morbidity and mortality in this age-group.

In conclusion, our findings suggest that non-household transmission of TB occurs at significant rates and warrants consideration of screening among young children in high TB incidence countries. Similar to many other settings, IPT uptake among children exposed to an infectious TB case is far below the global target of 90%, as set by WHO. Contact tracing of young children remains an important strategy to prevent active TB disease. To attain this, a road map to zero TB deaths among children emphasizes, among other issues, the collective responsibility of the health system, greater awareness among healthcare providers, and increased childhood TB screening (WHO, 2013). Integrating routine pediatric TB screening with child health clinics will enhance awareness to the community and advocate for early screening among young children suspected to have TB (Diese et al., 2016, Kancheya et al., 2014, Marais, 2017). In TB-endemic settings where TB exposure in the community is high, strategies to evaluate TB infection should not be limited to child contacts only.

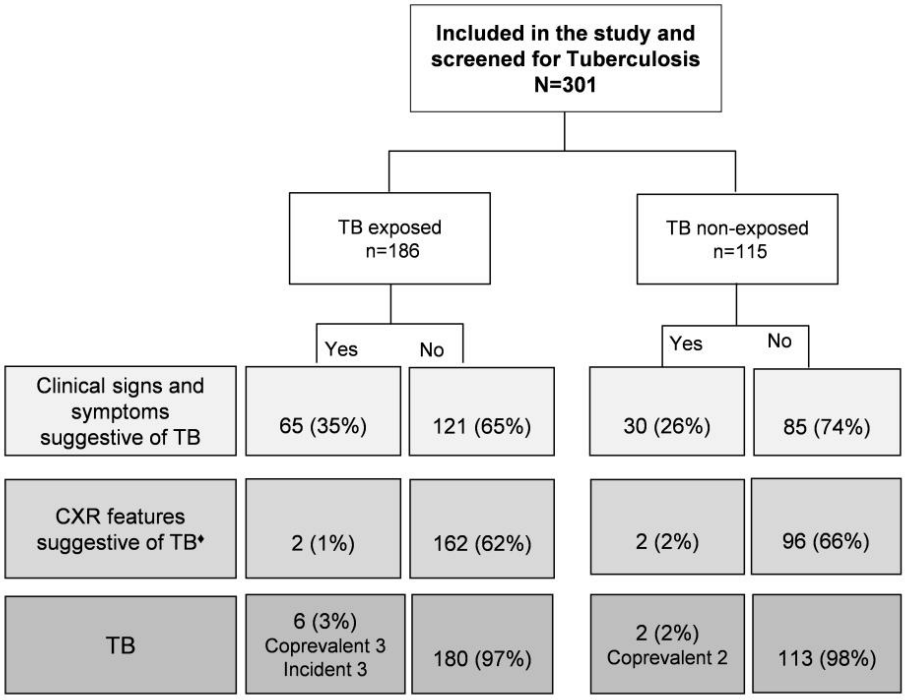
Manuscript II

Acknowledgements

We thank all parents/caregivers who allowed their children to participate in this study. We are grateful to the Temeke municipality, Temeke hospital, district and regional TB coordinators of the Temeke district, as well as the National TB and Leprosy Control Program in Tanzania for their support.

Manuscript II

Supplementary information



CXR, chest X-ray; TB, Tuberculosis

Clinical signs and symptoms suggestive of TB included: Current cough of any duration; Failure to thrive in the past three months; Fever: axillary temperature above 37.5ºC; Enlarged cervical lymph nodes of 2x2cm

♦262 children had chest x-ray done

Coprevalent TB cases: TB diagnosed with three months after study enrollment

Incident TB cases: TB diagnosed three months after study enrollment

Supplementary Figure 1. Screening algorithm and diagnosis of TB among enrolled children

Manuscript II

Supplementary Table 3. Determinants of quantiFERON (QFT) conversion and reversion at three-month follow-up visit among under-fives in Dar es Salaam, Tanzania

Characteristics	All n=226 (%)	Conversion (n=22)		Reversion (n=25)	
n (%)		aOR (95% CI)	p value	aOR (95% CI)	p value
Age groups (months)			0.96		0.89
6-24	104 (46)	1		1	
25-59	122 (54)	1.16 (0.35-3.84)		0.97 (0.41-2.27)	
Sex			0.25		0.21
Male	110 (49)	1		1	
Female	116 (51)	2.28 (0.61-8.48)		0.57 (0.24-1.33)	
TB exposure score [1]			0.18		0.32
No or low	87 (39)	1		1	
Medium or maximum	139 (61)	0.39 (0.08-1.85)		0.62 (0.27-1.46)	
Lymphocyte count [2]			0.40		0.31
Low	16 (7)	1		1	
Normal	175 (77)	0.16 (0.02-1.12)		2.24 (0.28-17.9)	
High	35 (16)	0.30 (0.03-2.70)		0.41 (0.02-7.01)	
Mother index case [3]			0.88		0.73
No	184 (81)	1		1	
Yes	42 (19)	1.10 (0.25-4.85)		0.86 (0.28-2.70)	
IPT during follow-up [4]			0.17		0.10
No	104 (46)	1		1	
Yes	122 (54)	0.49 (0.15-1.58)		1.45 (0.93-2.25)	
Helminth infection			0.25		0.22
No	171 (76)	1		1	
Yes	55 (24)	1.98 (0.60-6.59)		0.51 (0.16-1.57)	

75 children who did not come for follow-up visit were excluded in the analysis

[1] TB exposure score base on Mandalakas et. al. [11]. No exposure 0, Low exposure score, 1–6; medium/maximum, 7-10

[2] Lymphocytes: Low: <2.1; normal: 2.1-9.5, high: >9.5 $10^3/\mu$ l

[3] Applicable to TB-exposed group only based on TB exposure score based on Mandalakas et. al. [11]

[4] IPT: Isoniazid Preventive Therapy assessed at follow-up

Core multivariate model comprised of age, sex and lymphocyte count and adjusted for TB exposure score, mother index case, IPT during follow-up and helminth infection

Manuscript III

4. Impact of infections on Growth Development, Micronutrient Status and Cognitive function in Pre-School Aged Children in Tanzania

Khadija Said [1,2,3 *], Colin Cercamondi [4], Jerry Hella [1,2,3], Lujeko Kamwela [1,2,3], Rajesh Solank[5], Ramadhan Golly [5], Francis Mhimbira [1,2,3], Nicole Ritz [3,6], Stefanie Knopp [2,3], Christian Schindler [2,3], Anna M Mandalakas [7], Karim Manji [8], Marcel Tanner [2,3], Jürg Utzinger [2,3], Michael B. Zimmermann [4], Lukas Fenner [9 *]

[1] Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania, [2] Swiss Tropical and Public Health Institute, Basel, Switzerland, [3] University of Basel, Basel, Switzerland, [4] Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland, [5] Temeke Municipal Council Hospital, Dar es Salaam, Tanzania, [6] Basel University Children's Hospital (UKBB), Basel, Switzerland, [7] The Global Tuberculosis Program, Texas Children's Hospital, Department of Pediatrics, Baylor College of Medicine, Houston, United States of America, [8] Muhimbili University of Health and Allies Sciences, Dar es Salaam, Tanzania, [9] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

*Correspondence:
Emails: ksaid@ihi.or.tz (KS); lukas.fenner@ispm.unibe.ch (LF)

Conflicts of interest statement

All have none to disclose.

Funding

This work was supported by funding from the Rudolf Geigy Foundation (Basel, Switzerland). KS is a beneficiary of the Swiss Government Excellence Scholarship for Foreign Scholars and Artists (ESKAS).

Keywords: Child development, Cognitive, Deficiency, Infections, Under-resourced settings

Short Title: Coinfection and malnutrition in child development in Tanzania

To be submitted to Pediatric Infectious Disease Journal

Manuscript III

4.1. Abstract

Background: Malnutrition and infections are factors known to influence growth development and cognitive functioning of children and often co-exist in settings with inadequate sanitation, overcrowding and low socioeconomic status. We conducted a longitudinal study to assess the impact of infections on growth development, micronutrients and cognitive function among children under five years of age (under-five) in Dar es Salaam.

Methods: We enrolled 293 under-fives, 73% (214/293) completed six months of observation. We collected demographic, clinical, nutritional and anthropometry data and collected blood, stool, urine and sputum to screen for infections (helminths, tuberculosis, HIV, malaria) at enrollment and six-month follow-up visit. We used linear regression to analyze risk factors for change in nutritional status.

Results: We enrolled 293 children; median age was 26 months (interquartile range 17-42), one fifth of children were stunted and one fourth were wasted, 24% (70/293) had helminth infection, 22% (59/293) had tuberculosis infection. The prevalence of ferritin deficiency was 17% and 33% had vitamin A deficiency. There was no positive change in serum ferritin and soluble transferrin receptor after six months of follow-up. Children who were dewormed after recruitment had improved language cognitive score coefficient 0.04 (95% confidence interval [CI]-0.03-0.11) compared to others coef. -0.03 (95% CI-0.11-0.05), p=0.03. Children reported to have been receiving nutrient deficient meals were also observed to have improved language score 0.08 (95% CI 0.001-0.17, p=0.01). Vitamin A deficiency was not association with increased cognitive scores.

Conclusion: The observed improvement in micronutrient level and components of cognitive function score could be attributed to intervention at recruitment following diagnosis of the underlying causes. Infection and malnutrition screening interventions are vital to enable early management of childhood conditions for optimal growth development and cognitive function.

Manuscript III

4.2. Introduction

Infections negatively impacts nutritional status and are a leading cause of morbidity in younger children of less than five years of age (WHO, 2016b, WHO, 2017a, WHO, 2017b). Studies have shown helminth infections contribute to stunting and underweight. Helminth parasites cause malabsorption resulting to anorexia or compete for nutrients with its host. This happens in settings were populations are already on marginal diets subsequently leading to nutritional deficiency (Stephenson et al., 2001). Evidence suggests that micronutrients such as vitamin A, iron, copper, selenium, cobalt and zinc are reduced following helminth infection (Culha and Sangün, 2007, Arinola et al., 2015). Vitamin A is known to be essential vitamin for growth, vision and immune function (WHO, 2009). In responding to infections, host immune system requires increased energy consumption consequently increasing micronutrients requirements (Schaible and Kaufmann, 2007). Due to the pre-existing deficiency, cellular immune function is impaired increasing the risk for other infections such as *Mycobacterium tuberculosis* (*M. tuberculosis*), malaria, and respiratory viral infections (Jaganath and Mupere, 2012, Wessells et al., 2014, Paynter et al., 2014, Glinz et al., 2017).

Malnutrition affects growth and development, subsequently affecting cognitive performance of children (Ali, 2013). Stunting as an indicator of chronic malnutrition has been shown to be associated with poor psychomotor and mental development among infants in Tanzania (Mcdonald et al., 2013). Iron deficiency has been shown to affect memory and development even in the long-term (WHO, 2008). The co-existence of malnutrition and infection in young children substantially worsens infection outcome and prognosis which may contribute to increased mortality (Guerrant et al., 2008).

Due to existence of multiple risk factors such as malnutrition, micronutrient deficiency, infection and poor unstimulating environment, majority of children fail to reach their optimal cognitive development. Given their poor living conditions, children in such settings are at an increased risk of multiple infections, malnutrition and poor development of cognitive function, young children are highly likely to have poor prognosis (Akman et al., 2004, Gladstone et al., 2010, Righetti et al., 2013). We conducted a longitudinal study to assess the impact of infection on growth, micronutrient status and cognitive function among

children under-five years of age in a poorly planned urban district of Dar es Salaam, Tanzania.

4.3. Material and Methods

Study setting and study population

The study was conducted in Temeke district in Dar es Salaam, Tanzania between October 2015 and September 2016. Temeke is one of three districts in Dar es Salaam, Tanzania, has an estimated population of 1.4 million residents. We conducted a longitudinal study were we enrolled children 6-59 months of age with documented history of TB exposure and recruited their controls from neighboring households where no TB case was not documented. Enrolled children were followed-up for six month. Details have been previously described (Said et al., 2017a, Said et al., 2017b)

Study population

Among the 325 children whose parents consented to the study, 293 children had complete baseline data set for both clinical and laboratory results and 73% (214/293) of them completed six month follow-up visit (Figure 14).

Study procedures

At enrollment: Sociodemographic, socioeconomic information and medical history were collected and physical examination was performed. We assessed feeding practices among children using a modified dietary diversity score questionnaire that has a list of commonly consumed food in the past seven days. Weight, height and mid upper arm circumference measurements were performed using standard procedures. The study nurse assessed cognitive function of enrolled children using a validated Malawi Development Assessment Tool (MDAT) (Gladstone et al., 2010). Each child was assessed for 40 minutes. Parents or caregivers of acutely ill children were advised to return within a week of the child's recovery for assessment.

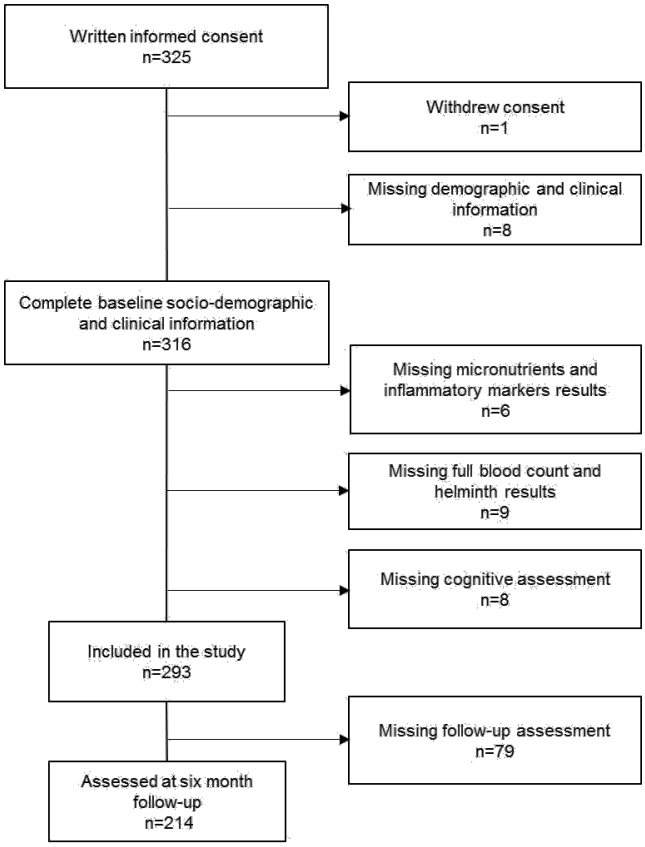


Figure 14. Selection of included children

Blood sampling procedures: We collected blood to analyze micronutrients and inflammatory markers. Four milliliters (ml) of blood for TB infection (LTBI) screening using QuantiFERON-TB Gold (QFT) ELISA analysis, full blood cell counts (FBC), malaria and HIV screening were also collected. We also collected plasma for zinc measurement, blood samples were collected at least 12 hours after the last meal and just before breakfast. Venous blood was drawn with zinc-free blood collection needles into S-Monovette heparinized zinc-free tubes. Blood was immediately centrifuged and plasma was transferred using plastic zinc-free pipet into zinc-free tubes and frozen at -20°C until analysis. For QFT, we collected an additional blood sample at three months to detect recent TB infections.

At the 6-month follow-up visit: We repeated clinical evaluation and cognitive assessment. We also repeated the blood collection at three month follow-up visit (3 ml of blood to repeat QFT analysis) and at six month follow-up visit to repeated blood collection and reanalyzed micronutrients and inflammatory markers. All

samples were received at the Temeke clinic and transported to the laboratory in appropriate temperature-controlled cooler boxes for testing procedures within five hours of sampling. Children found to have medical conditions or malnourished were given medical treatment and nutritional counseling in accordance with the Tanzanian guidelines (Said et al., 2017a).

Laboratory investigations

Blood Testing: We analyzed plasma zinc using AA240FS Fast Sequential Atomic Absorption Spectrophotometry at the Human Nutrition Laboratory at ETH Zurich University in Zurich. C-reactive protein (CRP), α_1-acid glycoprotein (AGP), Retinol Binding Protein (RBP), Ferritin, Iron stores and soluble transferrin receptor (sTfR) were analyzed using Sandwich ELISA technique machine in VitMin Lab, Willstaett, Germany. Due to noncompliance of sterile procedures at the time of enrollment, we therefore analyzed plasma zinc samples of follow-up visit only.

Other blood test: We screened for HIV using age appropriate test. We also performed malaria rapid diagnosis tests, FBC, QFT ELISA as previously described (Said et al., 2017b).

Helminth Investigation: One fresh stool sample and urine were collected at enrollment and six month follow-up visit from each child to screen for helminthes. Details previously described (Said et al., 2017a).

Data collection and definitions

We recorded clinical data directly onto tablet computers using the open-source software open data kit (ODK; http://opendatakit.org/), and the data management tool ―*odk_planner*. Laboratory results were transcribed onto ODK from laboratory results forms.

Micronutrient deficiency was defined as: follows Ferritin normal if adjusted serum ferritin ≥12 mg/L and deficient if <12 mg/L; vitamin A normal if adjusted RBP ≥0.75 µmol/L and deficient if <0.75 µmol/L. CRP elevated if >5 mg/L and normal if ≤5 mg/L; AGP elevated if >1 g/l and normal ≤1 g/L. Z-score were defined according to WHO. Helminth infection has been previously defined (Said et al., 2017a).

Manuscript III

Statistical analysis

Frequencies and proportions were used to describe the study population. A measure of socioeconomic status was derived using principal component analysis of household asset variables and divided into three quartiles with high income quartile as reference category. We adjusted RBP and ferritin values using correction factors considering different phases of infection: reference, incubation, early convalescence and late convalescence (Thurnham et al., 2003, Kung'u et al., 2009). We used medians to compare baseline and follow-up adjusted hemoglobin, serum ferritin and vitamin A values and plotted boxplots for visualization. Using a linear mixed regression model with random intercepts at the level of matched pairs, we analyzed association between change in adjusted serum iron, sTfR and vitamin A levels between baseline and six month follow-up with various risk factors, distinguishing children; a) without the respective risk factor at any of the two visits, b) no longer showing risk factor at follow-up, c) newly showing the risk factor at follow-up and d) showing the risk factor at both visits. For LTBI, the generated variable was based on baseline and three-month follow-up visit. We performed analogous linear mixed regression analyses to identify risk factors for changes in the four components of cognitive assessment tool considering the same risk factors as above, additionally adjusting for age, sex and socioeconomic status. Beeswarms plots were used to plot the correlation calculated from the baseline micronutrient levels and iron indices with inflammatory markers. All analyses were performed in Stata version 13.1 (Stata Corporation; College Station, United States of America).

Ethics statement

The study was approved by the Institutional Review Board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/No: 12-2015) and the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (NIMR; reference no. NIMR/HQ/R.8a/Vol. IX/2002) in Tanzania and the Ethics Committee of the north-west and central Switzerland (EKNZ reference no. UBE-15/49). All children were recruited after their parents/caregivers gave written informed consent.

Manuscript III

4.4. Results

Baseline patient characteristics

Among the 293 children at enrolment (baseline), the median age was 26 months (Interquartile Range [IQR], 17-42 months), 52% (152/293) were female; 66% (193/293) had anemia (hb <11g/dl), and 4% (13/293) had positive malaria rapid test results (Table 9). Over half of the children, 61% (178/293) were exposed to infectious smear-positive adult pulmonary TB cases. Overall, the median gross motor score was 0.72 (IQR 0.62-0.86) that was relatively lower than other three components of cognitive score: fine motor 0.82 (IQR 0.53-0.97), social 0.88 (IQR 0.73-0.96) and language 0.88 (IQR 0.75-0.97). Younger children, 6-24 months old, had a higher gross motor score coefficient 0.75 (95% CI0.72-0.77) than older children, 25-59 months 0.70 (95% CI 0.68-0.73), p=0.02.

Micronutrients, growth and cognition function at enrollment

Overall, 44% (128/293) children had micronutrient deficiency at the time of enrolment (baseline); 33% (96/293) children had vitamin A deficiency (adjusted retinol binding protein) and 17% (51/293) had serum ferritin deficiency. One fifth, 20% (59/293) of children were stunted, 25% were wasted was and 26% underweight. Younger children 6-24 months old had better gross motor score than older children 25-59 months coef. 0.75 (95% CI 0.72-0.77) *vs.* 0.70 (95% CI 0.68-0.73) p=0.020 but poor fine motor score coef. 0.62 (95% CI 0.59-0.64) *vs.* 0.86 (95% CI 0.83-0.89) p<0.001 and social scores coef. 0.78 (95% CI 0.74-0.80) *vs.* 0.86 (95% CI 0.84-0.89) p<0.001. There was no difference in language performance among the two age groups. Stunted children performed three out of four cognitive scores poorly than their peers; fine motor coef. 0.67 (95% CI 0.62 to 0.73) *vs.* 0.76 (95% CI 0.73 to 0.79) p=0.01 social coef. 0.73 (95% CI 0.69-0.77) *vs.* 0.85 (95% CI 0.83-0.87) p<0.001 and language coef. 0.77 (95% CI 0.73-0.81) *vs.* 0.85 (95% CI 0.83-0.87) p=0.014. Vitamin A deficiency showed no effect on cognitive function. Children with serum ferritin deficiency had lower fine motor and social score than their peers, coef. 0.68 (95% CI 0.61-0.73) *vs.* 0.76 (95% CI 0.73-0.79) p=0.0065, coef. 0.74 (95% CI 0.70-0.79) *vs.* 0.84 (95% CI 0.82-0.86) p<0.001.

Manuscript III

Table 9. Baseline demographic, anthropometric and laboratory characteristics in 293 children under-five years of age.

Characteristic	Value
Participants	293
Age (months), median, (IQR)	26 (17-42)
Female sex, n (%)	152 (52.0)
Anthropometry[1]	
HAZ mean, SD	-1.02 ± 1.35
Stunted, n (%)	59 (20)
WHZ mean, SD	-0.93 ± 1.58
Wasted, n (%)	73 (25)
WAZ mean, SD	-1.19 ± 1.38
Underweight, n (%)	76 (26)
Infection status n (%)	
Helminth	70 (24)
LTBI	37 (13)
Malaria	13 (4)
HIV	4 (1.4)
At least two infections	17 (6)
Anemia (hemoglobin <11.0 g/dl), n (%)	193 (66)
Micronutrients, median (IQR)	
Unadjusted[2] Ferritin (μg/l)	29.7 (15.7-54.8)
Deficient, unadjusted ferritin, n (%)	41 (14)
Deficient, ferritin[3], n (%)	149 (51)
Adjusted[4], Ferritin	24.9 (14.5-44.8)
Deficient adjusted ferritin, n (%)	51 (17)
Body iron stores (mg/kg bwt)	2.85 (-0.12- 5.58)
Reduced body iron stores, n (%)	75 (26)
Unadjusted RBP (μmol/l)	0.81 (0.67-0.96)
Deficient, unadjusted RBP, n (%)	110 (38)
Adjusted RBP (μmol/l)	0.85 (0.69-0.99)
Deficient, adjusted RBP, n (%)	96 (33)
Zinc[5] (μg/l)	92.9 (80.5-113)
Deficient Zinc, n (%)	25 (9)
Acute phase proteins, median (IQR)	
CRP (mg/l)	0.95 (0.37-4.91)
Elevated CRP, n (%)	73 (25)
AGP (g/l)	0.93 (0.63-1.61)
Elevated AGP, n (%)	141 (48)

[1] Anthropometrics are based on WHO Child Growth Standards median
HAZ: Stunting < –2 standard deviations (SD); WAZ: Underweight < –2SD; WHZ: wasting < –2SD, over weight > +2SD

[2] Before adjusting for inflammation

[3] WHO recommends cut-off <30μg/l as in settings with high inflammation

[4] Adjusted: After adjusting for inflammation (Thurnham et al., 2003, Thurnham et al., 2010); Unadjusted: Without adjusting for inflammation

[5] Sample collected at six-month follow-up visit

AGP: α -1-acid-glycoprotein, normal <1, elevated ≥1g/l; Body Iron store: low ≤0, normal >0mg/kg bwt; CRP: C- reactive protein, normal ≤5mg/l, >5 elevated; IQR, interquartile range; LTBI: Latent tuberculosis infection as determined by QuantiFERON-TB Gold; RBP: Retinol Binding Protein <0.75 low deficiency, 0.75-1.05 moderate deficiency, >1.05 normal level; Serum ferritin: low<12, normal ≥12 ug/l; SD, standard deviation
Helminth infection include: infection with *Ascaris lumbricoides*, *Enterobius vermicularis, Hymenolepis diminuta*, hookworms, *Schistosoma haematobium*, *Schistosoma mansoni*, *Strongyloides stercoralis*

The median hematological indices were not statistically significantly different between children with various infections and those without infection (Supplementary Table 4). The median serum ferritin levels were significantly high among children with raised acute phase protein (APP) (see Supplementary Figure 2).

Associations between infections, malnutrition and micronutrient recovery

There was a significant difference in median levels of Vitamin A levels between recruitment and follow up (Figure 14). Generally, there was a decrease of serum ferritin and soluble transferrin receptor (sTfR) levels among our study population except for children who were diagnosed with LTBI at enrollment but this was not statistically significant. The decrease of serum ferritin and sTfR levels was not association with age, sex, stunting, infections (helminth and malaria) as well as anemia as shown in Table 10. However, children who were reported to be fed nutrient deficient meals had a higher decrease of serum ferritin levels compared to children who were fed nutrient rich meals (coef. -16.4 [95% CI 25.1 to -7.66] *vs.* -5.21 [11.0 to -0.61], p=0.04). While children who were given anthelminthic after recruitment visits had less decrease of serum ferritin levels, children who did not take the anthelminthic had higher decrease of serum ferritin levels (coef. -3.90 [95% CI 9.84 -2.06] *vs.* -17.6 [-25.9 to -9.48], p=0.01). We observed a recovery of vitamin A level but had no significant association with any variable of interest including age, deworming and type of meal consumed (Table 10).

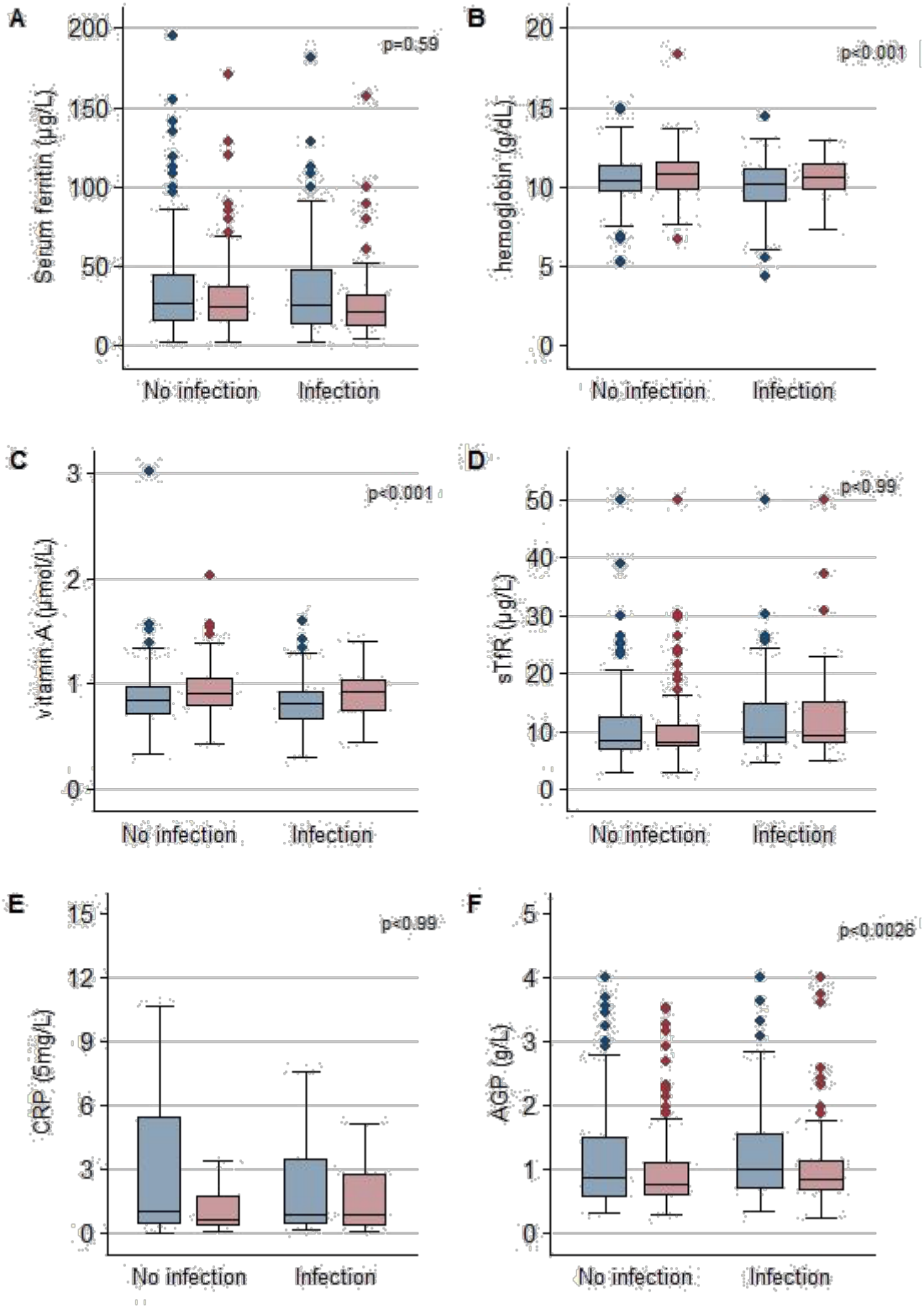


Figure 15. Figure 14. Box plots of baseline (left) and six month follow-up (right) micronutrient levels and inflammatory markers among 214 children under five years in Dar es Salaam, Tanzania.

Manuscript III

Table 10. Mean changes in micronutrient levels during six months of follow-up in 214 children according to infection and other patient-related factors

Variable	All 214 n (%)	Ferritin		sTfR		Vitamin A	
		Unadjusted Coef. (95% CI)	p value	Unadjusted Coef. (95% CI)	p value	Unadjusted Coef. (95% CI)	p
Sociodemographic and socioeconomic							
Age groups (months)			0.14		0.66		0
25-59	119 (56)	-5.31 (-11.9 -1.26)		-0.65 (-1.59-0.30)		0.05 (-0.002-0.11)	
6-24	95 (44)	-12.7 (-20.0 to -5.49)		-0.33 (-1.39-0.74)		0.10 (0.03-0.16)	
Sex			0.44		0.43		0
Female	108 (50)	-10.6 (-17.4 to -3.71)		-0.31 (-1.53-0.93)		0.10 (0.04-0.16)	
Male	106 (50)	-6.69 (-13.6-0.28)		-0.79 (-1.80-0.22)		0.05 (-0.01-0.11)	
SES			0.24		0.74		0
Low	80 (38)	-4.48 (-13.0- 4.09)		-0.19 (-1.70-1.32)		0.06 (-0.01-0.14)	
Middle	65 (30)	-14.0 (-22.9 to -6.00)		-0.86 (-2.02-0.30)		0.08 (0.01-0.15)	
High	69 (32)	-6.57 (-15.4-2.26)		-0.29 (-1.55-0.98)		0.07 (-0.04-0.18)	
Anthropometry							
Stunting			0.71		0.90		0
Not stunted	141 (66)	-7.74 (-13.8 to -1.70)		-0.42 (-1.29-0.45)		0.08 (0.02-0.13)	
Improved at FU	16 (7)	-5.66 (-23.4-12.1)		-0.65 (-3.19-1.89)		0.10 (0.02-0.12)	
Became stunted at FU	28 (13)	-7.37 (-20.8-6.0)		-0.21 (-2.14-1.71)		0.03 (-0.09-0.14)	
Stunted at both visits	29 (14)	-15.9 (-29.1 to -2.72)		-1.13 (-3.02-0.76)		0.09 (-0.02-0.21)	
Infections							
Helminth infection			0.08		0.88		0
Not infected	129 (60)	-7.95 (-14.2 to -1.74)		-0.52 (-1.44-0.40)		0.09 (-0.004-0.19)	
Not infected at FU	29 (14)	-10.8 (-23.8-2.25)		-0.54 (-2.45-1.37)		0.10 (-0.05-0.25)	
Became infected at FU	38 (18)	-0.57 (-12.1-10.9)		-0.84 (-2.50-0.82)		0.05 (-0.08-0.19)	
Infected at both visits	18 (8)	-26.9 (-43.4 to -10.4)		0.37 (-2.12-2.76)		0.12 (-0.05-0.28)	
Latent TB infection			0.23		0.88		0
Negative at enrollment	163 (76)	-10.8 (-16.4 to -5.26)		-0.55 (-1.37-0.27)		0.06 (0.02-0.11)	
Positive at enrollment	13 (6)	4.42 (-15.1-23.9)		-0.12 (-2.69-2.94)		0.13 (-0.04-0.30)	
Converted to positive at FU	18 (8)	-11.1 (-27.8-5.49)		-0.04 (-2.39-2.48)		0.17 (0.02-0.32)	
Reverted to negative at FU	20 (10)	2.92 (-13.2-19.1)		-1.08 (-3.37-1.21)		0.03 (-0.11-0.17)	
Malaria			0.76		0.32		0
Negative	206 (96)	-8.51 (-13.5 to -3.52)		-0.43 (-1.17-0.30)		0.07 (0.03-0.12)	
Positive	8 (4)	-12.4 (-37.6-12.7)		-2.30 (-5.89-1.28)		0.07 (-0.15-0.29)	

Manuscript III

Variable	All 214 n (%)	Ferritin		sTfR		Vitamin A	
		Unadjusted Coef. (95% CI)	p value	Unadjusted Coef. (95% CI)	p value	Unadjusted Coef. (95% CI)	p
Nutritional factors							
Anemia (<11.0g/dl)			0.22		0.04		0
Not anemic	54 (25)	-1.71 (-11.4-7.96)		-0.39 (-0.98-1.77)		0.09 (0.003-0.17)	
Improved at FU	40 (19)	-17.4 (-28.5 to -6.23)		-2.52 (-4.13 to -0.91)		0.06 (-0.04-0.16)	
Became anemic at FU	17 (8)	-6.9 (-24.0-10.14)		-0.18 (-2.58-2.23)		-0.01(-0.16-0.14)	
Anemic at both visits	103 (48)	-9.05 (-16.2 to -2.15)		-0.25 (-1.25-0.75)		0.08(0.02-0.15)	
Consumed food			0.04		0.99		0
Nutrients rich	149 (70)	-5.21 (-11.0 to -0.61)		-0.51 (-1.36-0.35)		0.06(0.01-0.11)	
Nutrient deficient	65 (30)	-16.4 (-25.1 to -7.66)		-0.50 (-1.78-0.78)		0.11(0.04-0.19)	
Deworming medication[1]			0.01		0.37		0
No deworming	73 (35)	-17.7 (-25.9 to -9.48)		-0.01 (-1.19-1.16)		0.05 (-0.02-0.12)	
Albendazole/mebendazole	138 (65)	-3.90 (-9.85-2.06)		-0.67 (-1.53-0.18)		0.09 (0.03-0.14)	

Change in adjusted ferritin/ sTfR/Vitamin A levels= value at six month visit minus baseline value

Categories were mutually exclusive

Consumed food: Based on dietary diversity score, (deficient 1-6 and rich if 7-10); LTBI: Latent TB infection as determined by QuantiFERON-TB Gold; SES: Socio-economic status; sTfR: soluble transferrin receptor; HAZ: Stunting < –2 standard deviations (SD); WAZ: Underweight < –2SD; WHZ: wasting < –2SD, over weight > +2SD

Helminth species in children infected at both visits were: *Schistosoma mansoni* 72% (13/18), *Strongyloides stercoralis* 22% (4/18), *Schistosoma haematobium* 6% (1/18), *Ascaris lumbricoides* 6% (1/18) and *Hymenolepis diminuta* 6% (1/18); follow-up: *S. mansoni* 61% (11/18), hookworms 17% (3/18), *S. stercoralis* 11% (2/18), *Enterobius vermicularis* 6% (1/18) and *S. haematobium* 6% (1/18)

[1] Parents of 211 children responded to deworming medication question

Manuscript III

Associations between infection, growth development and development cognitive function

At the end of six month of follow-up, all four components of cognitive function assessment showed improvement in relation to different variables (Table 11). Younger children, 6-24 months old were more likely to have improved score of two out of the four cognitive components compared to older children: fine motor score coef.0.27 (95% CI 0.20-0.33, p=<0.001) and social score 0.12 (95% CI 0.07-0.17, p=<0.001) shown in Table 11 below. Anthropometric growth correlated with influence on social cognitive score; though children who did not present with stunting at follow up had positive coef. 0.07 (95% CI -0.02-0.17), children who were stunted at both visits had higher increase in coef. 0.11 (95% CI 0.04-0.19, p=0.003). Children who were underweight during the two study visits had higher change compared to others coef. 0.10 (95% CI 0.03-0.17, p=0.002).

Helminth infection was shown to affect gross and language components: children diagnosed with helminth infection at baseline and follow-up visit had highest gross motor cognitive score change compared to others coef. 0.19 (0.07-0.31) p=0.03 and language change 0.13 (95% CI 0.02-0.25, p=0.04). Malaria and LTBI did not show any significance with cognitive improvement across the four components. Nutritional factors such as anemia, serum ferritin, vitamin A and nutritional content of the food consumed by children showed influence on cognitive development: children who were anemic at both visits had higher increase of gross motor coef. 0.17 (95% CI -0.09-0.26, p=0.04) and fine motor coef. 0.15 (95% CI 0.07-0.23, p=0.02) compared to all others. Children who were no longer vitamin A deficient at six month follow-up visit had a higher improvement of vitamin A levels than others coef. 0.15 (95% CI 0.06-0.24, p=0.045). Children whose parent/caregivers reported nutrient deficient meals during recruitment had increased language scores during follow-up, coef. 0.08 (95% CI 0.001-0.17) while their peers, had decreased their scores coef. -0.01 (95% CI -0.07-0.06), p=0.01. Children who were dewormed within three months after recruitment into the study had higher recovery of language score compared to their peers who were not dewormed coef. 0.04 (95% CI -0.03-0.11, p=0.03).

Manuscript III

Table 11. Mean changes in cognitive score components during six months of follow-up in 214 children according to infection and other patient-related factors

Variable	All 214 n (%)	Cognitive components							
		Gross motor		Fine motor		Social		Language	
		Adjusted Coef. (95% CI)	p value	Adjusted Coef. (95% CI)	p value	Adjusted Coef. (95% CI)	p value	Adjusted Coef. (95% CI)	p value
Sociodemographic and socioeconomic									
Age groups (months)			0.097		<0.001		<0.001		0.30
25-59	116 (55)	0.13 (0.06-0.20)		0.07 (0.01-0.14)		0.02 (-0.03-0.07)		0.02 (-0.05-0.08)	
6-24	95 (45)	0.08 (0.01-0.14)		0.27 (0.20-0.33)		0.12 (0.07-0.17)		0.05 (-0.02-0.11)	
Sex			0.44		0.86		0.22		0.62
Female	107 (51)	0.13 (0.06-0.20)		0.07 (0.01-0.14)		0.02 (-0.03-0.07)		0.04 (-0.05-0.08)	
Male	104 (49)	0.11 (0.04-0.18)		0.06 (0.0002-0.13)		0.05 (-0.0003-0.10)		0.03 (-0.04-0.10)	
SES			0.54		0.82		0.12		0.81
Low	79 (38)	0.16 (0.09-0.23)		0.08 (0.02-0.15)		0.0001 (-0.05-0.05)		0.02 (-0.04-0.09)	
Middle	64 (30)	0.13 (0.06-0.20)		0.07 (0.01-0.14)		0.02 (-0.03-0.07)		0.02 (-0.05-0.08)	
High	68 (32)	0.17 (0.10-0.24)		0.10 (0.03-0.16)		-0.04 (-0.09-0.01)		0.04 (-0.03-0.11)	
Anthropometry									
Stunting			0.93		0.10		0.003		0.06
Not stunted	141 (66)	0. 12 (0.05-0.20)		0.05 (-0.02-0.11)		-0.01 (-0.05-0.05)		-0.01 (-0.08-0.06)	
Improved at FU	16 (7)	0.16 (0.03-0.28)		0.15 (0.03-0.27)		0.07 (-0.02-0.17)		0.04 (-0.07-0.16)	
Became stunted at FU	28 (13)	0.11 (-0.003-0.23)		0.05 (-0.06-0.16)		-0.01 (-0.10-0.08)		-0.01 (-0.12-0.10)	
Stunted at both visits	29 (14)	0.14 (0.04-0.25)		0.13 (0.03-0.23)		0.11 (0.04-0.19)		0.11 (0.01-0.21)	
Wasting			0.26		0.08		0.96		0.78
Not wasted	133 (63)	0.10 (0.02-0.18)		0.05 (-0.02-0.13)		0.02 (-0.04-0.08)		0.02 (-0.06-0.09)	
Improved at FU	37 (18)	0.17 (0.08-0.26)		0.06 (-0.02-0.15)		0.03 (-0.04-0.10)		-0.001 (-0.09-0.09)	
Became wasted at FU	22 (10)	0.13 (0.01-0.24)		0.10 (-0.01-0.21)		0.04 (-0.05-0.12)		0.06 (-0.05-0.17)	
Wasted at both visits	19 (9)	0.17 (0.06-0.30)		0.15 (0.04-0.27)		0.02 (-0.08-0.11)		-0.001 (-0.12-0.12)	
Underweight			0.17		0.12		0.002		0.08
Not underweight	130 (62)	0.11 (0.04-0.19)		0.04 (-0.04-0.11)		-0.02 (-0.08-0.03)		-0.02 (-0.10-0.05)	
Improved at FU	30 (14)	0.20 (0.10-0.30)		0.12 (0.03-0.22)		0.06 (-0.02-0.13)		0.03 (-0.06-0.13)	
Became underweight at FU	20 (9)	0.17 (0.05-0.30)		0.04 (-0.08-0.16)		0.0001 (-0.09-0.09)		0.01 (-0.10-0.13)	
Underweight at both visits	31 (15)	0.09 (-0.07-0.19)		0.11 (0.02-0.21)		0.10 (0.03-0.17)		0.09 (-0.003-0.19)	
Infections									
Helminth infection			0.03		0.31		0.07		0.04
Not infected	127 (60)	0.15 (0.07-0.22)		0.07 (0.003-0.14)		0.01 (-0.05-0.06)		0.01 (-0.06-0.08)	
Not infected at FU	29 (14)	0.06 (-0.06-0.17)		0.03 (-0.08-0.13)		-0.02 (-0.10-0.07)		-0.03 (-0.14-0.08)	

Became infected at FU	37 (18)	0.05 (-0.05-0.14)		0..04 (-0.06-0.13)		0.04 (-0.04-0.11)	-0.03 (-0.14-0.05)	
Infected at both visits	18 (8)	0.19 (0.07-0.31)		0.14 (0.02-0.26)		0.11 (0.02-0.21)	0.13 (0.02-0.25)	
Malaria			0.05		0.67		0.16	0.17
Negative	203 (96)	0.14 (0.07-0.21)		0.07 (0.01-0.14)		0.03 (-0.03-0.08)	0.02 (-0.05-0.09)	
Positive	8 (4)	-0.03 (-0.2-0.15)		0.04 (-0.130.20)		-0.07 (-0.20-0.07)	-0.09 (-0.26-0.07)	
Latent TB infection			0.69		0.08		0.93	0.09
Negative at enrollment	161 (76)	0.12 (0.05-0.19)		0.08 (0.01-0.15)		0.03 (-0.03-0.08)	0.02 (-0.05-0.09)	
LTBI at enrollment	13 (6)	0.13 (-0.002-0.27)		-0.07 (-0.20-0.06)		-0.01 (-0.11-0.10)	-0.10 (-0.23-0.03)	
Converted to LTBI at FU	18 (8)	0.19 (0.07-0.31)		0.13 (0.02-0.24)		0.03 (-0.07-0.12)	0.10 (-0.01-0.22)	
Reverted to negative at FU	20 (9)	0.11 (-0.02-0.23)		0.12 (-0.001-0.23)		0.04 (-0.06-0.13)	0.03 (-0.08-0.15)	
Nutritional factors								
Anemia (<11.0g/dl)			0.04		0.02		0.18	0.42
Not anemic	53 (25)	0.12 (0.03-0.20)		0.04 (-0.04-0.11)		0.004 (-0.06-0.07)	-0.002 (-0.08-0.08)	
Improved at FU	40 (19)	0.05 (-0.05-0.15)		0.06 (-0.04-0.15)		0.001 (-0.07-0.08)	-0.01 (-0.11-0.08)	
Became anemic at FU	17 (8)	0.16 (0.04-0.28)		0.04 (-0.08-0.15)		0.02 (-0.08-0.11)	0.03 (-0.09-0.15)	
Anemic at both visits	101 (48)	0.17 (0.09-0.26)		0.15 (0.07-0.23)		0.6 (-0.003-0.13)	0.05 (-0.03-0.13)	
Serum ferritin			0.63		0.02		0.53	0.84
Normal	151 (72)	0.13 (0.06-0.20)		0.06 (-0.004-0.13)		0.02 (-0.03-0.07)	0.01 (-0.05-0.08)	
Improved	20 (9)	0.10 (-0.03-0.23)		0.005 (-0.11-0.12)		-0.01 (-0.11-0.08)	-0.01 (-0.13-0.11)	
Became deficient at FU	27 (13)	0.16 (0.04-0.28)		0.14 (0.03-0.25)		0.004 (-0.09-0.08)	0.05 (-0.07-0.16)	
Deficient at both visits	13 (6)	0.19 (0.05-0.34)		0.21 (0.08-0.35)		0.07 (-0.04-0.18)	0.04 (-0.10-0.17)	
Vitamin A			0.80		0.04		0.14	0.26
Normal	121 (57)	0.12 (0.04-0.19)		0.04 (-0.03-0.24)		-0.001 (-0.06-0.06)	-0.01 (-0.08-0.06)	
Improved	45 (21)	0.13 (0.04-0.22)		0.15 (0.06-0.24)		0.06 (-0.01-0.13)	0.01 (-0.08-0.10)	
Became deficient at FU	21 (10)	0.14 (0.02-0.27)		0.10 (-0.01-0.22)		0.07 (-0.03-0.16)	0.07 (-0.05-0.19)	
Deficient at both visits	24 (12)	0.17 (0.06-0.27)		0.06 (-0.04-0.17)		0.04 (-0.04-0.12)	0.07 (-0.03-0.17)	
Consumed food			0.46		0.06		0.12	0.01
Nutrients rich	146 (69)	0.12 (0.05-0.20)		0.05 (-0.01-0.12)		0.01 (-0.04-0.07)	-0.01 (-0.07-0.06)	
Nutrient deficient	65 (31)	0.15 (0.06-0.24)		0.12 (0.04-0.20)		0.05 (-0.01-0.12)	0.08 (0.001-0.17)	
Deworming medication[1]			0.10		0.63		0.19	0.03
No deworming	73 (35)	0.09 (0.01-0.18)		0.06 (-0.02-0.14)		-0.001 (-0.07-0.06)	-0.03 (-0.11-0.05)	
Albendazole/mebendazole	138 (65)	0.15 (0.08-0.22)		0.08 (0.01-0.15)		0.03 (-0.02-0.09)	0.04 (-0.03-0.11)	

Change in cognitive score = score at six month visit minus baseline value

Models adjusted for age, sex, and SES. P values refer to the differences in the estimated mean changes across the respective sub-categories of children

Consumed food: Based on dietary diversity score, deficient 1-6 and rich if 7-10; LTBI: Latent TB infection; SES: Socio-economic status; HAZ: Stunting < –2 standard deviations (SD); WAZ: Underweight < –2SD; WHZ: wasting < –2SD, over weight > +2SD

Helminth species in children infected at both visits were: *S. mansoni* 72% (13/18), *S. stercoralis* 22% (4/18), *S. haematobium* 6% (1/18), *A. lumbricoides* 6% (1/18) and *H. diminuta* 6% (1/18); follow-up: *S. mansoni* 61% (11/18), hookworms 17% (3/18), *S. stercoralis* 11% (2/18), *E. vermicularis* 6% (1/18) and *S. haematobium* 6% (1/18)

[1] Parents/caregivers of 211 children responded to the question on deworming medication

4.5. Discussion

We are presenting findings from a study where we assessed the impact of helminth and latent TB infections on growth, micronutrients and cognitive functioning in children under the age of five years for the six month of follow-up. We found high prevalence of anemia and vitamin A deficiency among study participants. Although there was an increase in vitamin A levels at the end of the observation, this mean change had no association with sociodemographic, anthropometry and nutritional risk factors. We are also reporting an improvement of all four of the cognitive function score. Both infections, micronutrients and deworming had an effect in the increase of cognitive performance.

Our results show high prevalence of anemia that was about four times the prevalence of serum ferritin deficiency. The reported prevalence was slightly higher than that reported in the recent Tanzania Demographic and Health Survey and Malaria Indicator Survey of 2015-16; that was 60% (Ministry of Health Community Development Gender Elderly and Children et al., 2016). Our prevalence was lower than that reported from the southern coastal region of Mtwara which assessed anemia among hospitalized under-fives (Mghanga et al., 2017). We recruited relatively healthy under-fives from the community. There was generally no positive change in serum ferritin; and sTfR from enrolment to six month follow-up visit, children who no longer presented with anemia also showed negative association with the mean change of ferritin levels, similar results of negative association of hemoglobin and serum ferritin was reported in Côte d'Ivoire (Righetti et al., 2013). Our results suggest that serum ferritin deficiency was not a major cause of anemia among our study population.

We report one third of children to have vitamin A deficiency (VAD) that had no association with any infection as well as anthropometric parameters. The reported deficiency is similar to that of TDHS of 2010 (National Bureau of Statistics et al., 2011). Similar high prevalence of VAD that is also of public health concern has been reported from Bangladeshi, Ethiopia, Kenya, Nigeria and South Africa ranging from 15-35% (Ahmed et al., 2016, Harika et al., 2017).

Manuscript III

In Tanzania, vitamin A supplementation is routinely done at reproductive and child health clinic (RCH) among children under the age of five years twice yearly from the age of six months, an initiative made by the country's Ministry of Health together with the support from the United Nations Children Fund (UNICEF) since 2001; that is coordinated by the Food and Nutrition Centre (TFNC) (Masanja et al., 2008). Vitamin A is essential for growth, vision and immune function and its deficiency is linked to increased morbidity and mortality (Hurwitz et al., 2017). Our study did not show association between VAD and helminth infection; our study participants had VAD but had low prevalence of *A. lumbricoides*. *A. lumbricoides* infestation is known to be associated with VAD. The observed deficiencies are likely due to low intake in the diet. Children from resource limited settings are faced with intake of meals with poor nutrient density as they do not have access to vitamin A rich foods such as animal products and fortified foods, their diets mostly consist of vegetables.

We report improvement of the mean cognitive development components that was influenced by improved diet intake, micronutrients levels, deworming, infection and anemia. All the reported factors have previously been shown to influence child growth, intellectual and cognitive development (Sakti et al., 1999, Jukes et al., 2002, Ezeamama et al., 2005, Grantham-Mcgregor et al., 2007). Children with anemia, others with underweight and those with helminth infection at two assessed visits showed higher mean change of gross, fine and social scores compared to others. Our findings show that, *S. mansoni*, and hookworms were among helminth species diagnosed among the 18 children with helminth at both visits. Chronic helminth infections have previously been shown to be associated with malnutrition, micronutrient deficiency and iron deficiency anemia from a combination of mechanism which involves intestinal obstruction and blood loss (Ali, 2013). Serum ferritin deficiency is also known to affect growth and cognitive development (Ibrahim et al., 2017). In our study, children without micronutrient deficiencies presented with small change in cognitive function in the six months of observation. Deworming was also associated with an increase in cognitive score. The positive change in those children could be due to the fact that, children found to have various medical conditions were given appropriate treatment. We provided medical treatment to all children

as per Tanzania's guidelines. The reported improvement may be a result of medical treatment and nutritional counseling provided. Lack of improvement in the other children (who did not present with any medical condition) can be explained by lack of intervention of nutritional counseling given to the other group. It has previously been shown that, deworming, together with other interventions improve cognitive function of children (Nokes et al., 1992, Nga et al., 2011, Lobato et al., 2012, Vaivada et al., 2017).

The co-existence of infections, specifically helminth, micronutrient deficiencies, high prevalence of poor nutritional indicators such as stunting and wasting, among under-five population who are at critical age of both physical and mental development (Walker et al., 2007), should be a matter of concern. Tanzania reported to have succeeded in reducing under-five mortality due to preventive interventions (Afnan-Holmes et al., 2015). Vitamin A supplementation is known to reduce morbidity and mortality (Imdad et al., 2017). Regular deworming is a cost effective helminth control intervention (Olds, 2013). Tanzania was faced with high prevalence of iodine deficiency; it has now reduced prevalence by successfully implementing iodine fortification of table salt. This has resulted in increased access of iodine to almost 90% in the households and has been the most common method of preventing iodine deficiency (Assey et al., 2009, Ministry of Health Community Development Gender Elderly and Children et al., 2016). Iodine is also an essential element in growth and cognition (Zimmermann, 2011) and so are vitamin A, ferritin and zinc. Another success story in the country is that of oral rehydration therapy with supplementation of zinc during childhood diarrhea management. This has resulted in significant low mortality from diarrhea in under-fives, another leading cause of morbidity and even mortality. Recently, the Ministry of Health has rolled out rotavirus vaccination within the expanded program on immunization that has been shown to be cost effective (Ruhago et al., 2015). This will result into further reduction of morbidity and mortality from childhood diarrhea illness. To increase the uptake of vital vitamin and other supplements, availability of fortified food for younger children should be considered. Food fortification will ensure high coverage, availability and better access of iron, vitamin A and other necessary supplements to this younger population in need of the essential micronutrients for optimal growth, development and cognitive functioning.

Manuscript III

In conclusion, the high prevalence of micronutrient deficiency is a matter of concern; it impairs children development and cognitive function. The co-existence of infections in these poor communities further worsens children potential. The Ministry of Health should consider use of child health clinics that are used for growth and development monitoring of under-fives as an avenue for sensitization and monitoring of micronutrients. Designing a user friendly assessment tool for use by lower health care workers to facilitate tailored interventions at primary health care facilities will assist in addressing micronutrient deficiencies. In order to avoid lifelong consequences, these interventions must target the early 1000 days of life if we are to improve the public health (Cunha et al., 2015). Specially, early intervention is key in ensuring children attain their optimal growth in their early years of life to maximize the quality of life saved ahead.

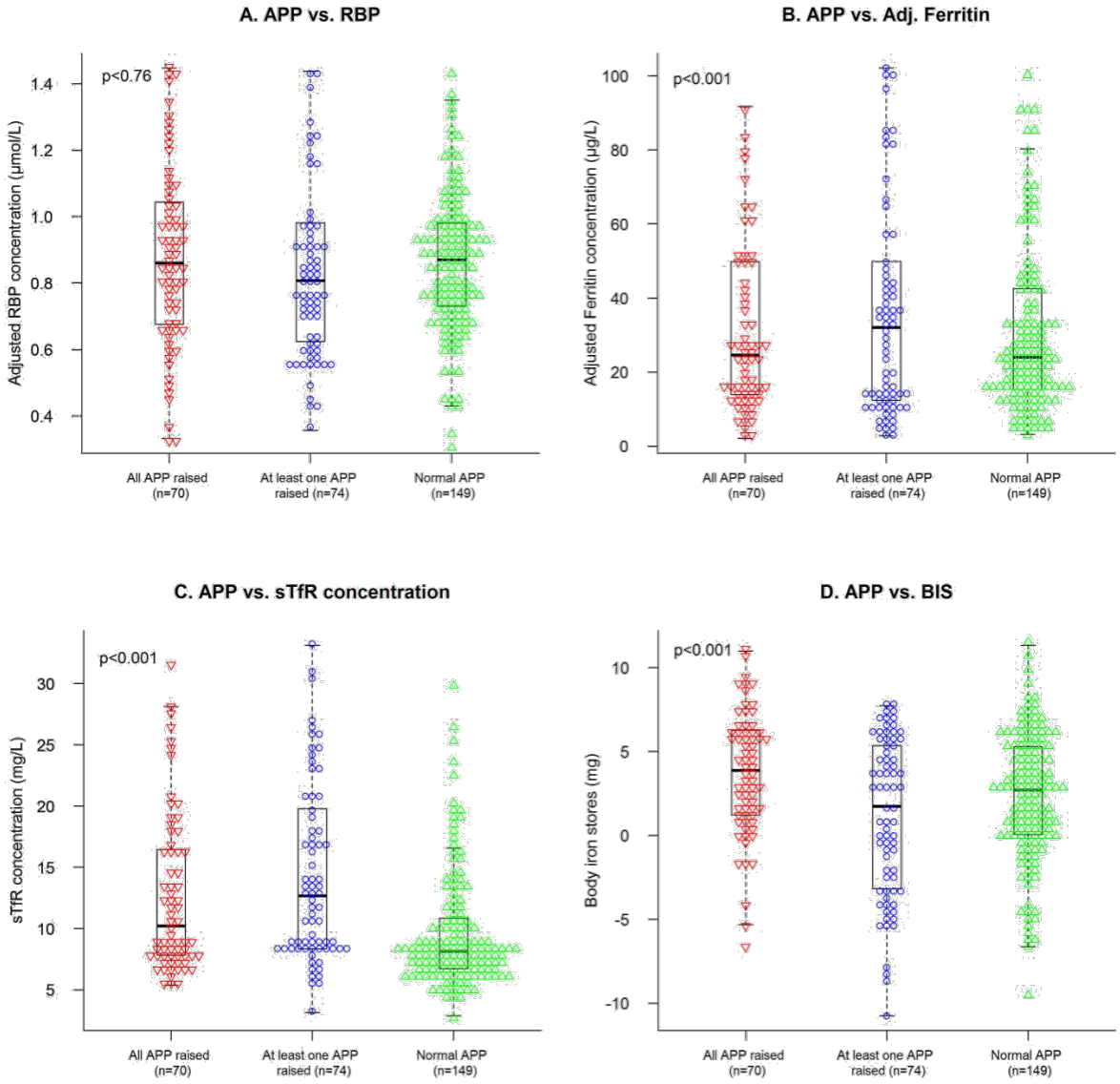
Manuscript III

Supplementary Information

Supplementary Table 4. Comparison of APP, hemoglobin, iron indices and red blood cell indices with infection in 293 children. with and without infections.

Iron status indicators/ RBC differentials	All children n=293	No infection n=186	Infections				P value
			TB n=25	Malaria n= 9	Helminth n=55	Coinfections n=17	
CRP (mg/l)	0.95 (0.37-4.91)	0.98 (0.36-5.11)	0.75 (0.38-2.65)	2.1 (1.12-9.1)	1.12 (0.45-4.1)	0.69 (0.28-4.49)	0.86
AGP (g/l)	0.93 (0.63-1.61)	0.89 (0.60-1.61)	1.01 (0.57-1.75)	1.04 (0.82-1.48)	0.98 (0.71-1.56)	1.43 (0.7-2.17)	0.62
Hb (g/dl)	10.4 (9.3-11.3)	10.4 (9.6-11.4)	10.9 (9.2-11.8)	10.3 (9.2-11.7)	10.2 (9.3-10.8)	10.2 (9.0-11.1)	0.44
Body Iron store (mg/kg bwt)	2.8 (-0.12-5.58)	2.9 (-0.14-5.64)	2.72 (-0.17-5.52)	2.9 (1.98-4.69)	2.52 (-0.12-5.65)	2.16 (1.01-5.42)	0.83
Serum ferritin (μg/L)	29.7 (15.7-54.8)	24.1 (14.6-43.1)	29.0 (12.4-41.4)	31.3 (19.7-52.3)	26.3 (15.7-57.0)	24.9 (14.8-66.3)	0.85
sTfR (μg/l)	8.88 (7.3-14.26)	8.77 (7.04-14.0)	8.45 (6.7-13.9)	11.5 (9.74-14.8)	9. 54 (7.84-13.6)	11.7 (7.58-16.5)	0.18
MCV (fL)	69.0 (63.0-74.0)	70.0 (63.0-75.2)	68.1 (66.8-73.3)	67.8 (64.0-70.0)	66.0 (60.1-73.7)	67.4 (58.1-73.0)	0.13
MCH (μg/cell)	22.2(19.7-24.6)	22.2 (20.1-24.7)	22.6 (21.0-24.8)	20.2 (19.6-21.8)	21.5 (18.7-23.5)	21.4 (18.3-25.2)	0.086
MCHC (g/dl)	32.3 (30.3-33.9)	32.3 (30.5-34.0)	33.0 (31.9-33.8)	29.7 (29.1-31.3)	31.7 (29.6-33.8)	31.6 (31.6-34.6)	0.031

Median values (interquartile range, IQR) are shown. P values were derived from kruskal-wallis test.
AGP: α -1-acid-glycoprotein, normal <1, elevated ≥1g/l; Body Iron store: low ≤0, normal >0mg/kg body weight; CRP: C- reactive protein, normal ≤5mg/l, >5 elevated; Hb, Hemoglobin level, low <11g/dL, normal ≥11g/dL; MCV: mean corpuscular volume, low<56.4, normal 56.4-83.1, high >83.1 fL ; MCH: mean corpuscular hemoglobin, low<17.5, normal 17.5-28.5, high >28.5 pg/cell; MCHC: mean corpuscular hemoglobin concentration low<30.1, normal 30.1-35.6, high >35.6 g/dL; RBP: Retinol Binding Protein <0.75 low deficiency, 0.75-1.05 moderate deficiency, >1.05 normal level; Serum ferritin: low<12, normal ≥12 ug/l; sTfR; Soluble Transferrin Receptor, normal ≤8.3, elevated >8.3μg/l



APP: Acute Phase Proteins

Body Iron store: low ≤0, normal >0mg/kg bwt; RBP: Retinol Binding Protein <0.75 low deficiency, 0.75-1.05 moderate deficiency, >1.05 normal level; Serum ferritin: low<12, normal ≥12 ug/l; sTfR; Soluble Transferrin Receptor, normal ≤8.3, elevated >8.3µg/l

Supplementary Figure 2. Graphs showing correlation of baseline micronutrient levels and iron indices with inflammatory markers among 293 children under five years in Dar es Salaam, Tanzania.

5. Discussion

The overall aim of this PhD thesis was to generate better understanding on the co-existence of helminth and *M. tuberculosis* co-infections and its impact on growth, nutritional status and cognitive function in the most vulnerable population of children under the age of five years (under-fives) particularly, focusing on the urban setting of Dar es Salaam, Tanzania. The project took advantage of an ongoing Tuberculosis Cohort Study in the Dar es Salaam region (TB-DAR). The specific objectives of the PhD thesis were: (i) to determine the prevalence and intensity of helminth infections among children with and without documented exposure to infectious TB patients; (ii) to assess the prevalence of TB infection by QFT among children exposed and unexposed to infectious TB case, and to evaluate the screening strategy used and; (iii) to assess the impact of helminth and *M. tuberculosis* infections on growth, micronutrient status and cognitive function among children in a poorly planned area of Temeke district in Dar es Salaam. In the following section we will summarize and discuss the principal findings of this thesis, identified gaps, highlight limitations and suggest further studies to test and confirm our findings.

5.1. Summary of the principal findings

The findings from our cross-sectional study that assessed the prevalence of helminth infection among under-fives show a surprisingly high estimate of POC-CCA positive that might indicate a high prevalence of *S. mansoni* among other helminth infections. This high prevalence of *S. mansoni* (as determined by POC-CCA) lacked a significant association with commonly reported risk factors such as age, sex, poor hygiene and social economic status. In regard to associated symptoms at baseline, overall, we found helminth infection to be associated with anemia but did not influence child development and cognitive function. The first longitudinal study that assessed the prevalence of latent TB infection (LTBI) among under-fives with and without documented exposure to an infectious TB also showed surprising results. The prevalence of LTBI in the two groups was similar. Children from households with up to five people were at higher risk of having LTBI compared with children who came from relatively larger households.

Discussion

The uptake of IPT among children documented to have TB exposure was also shown to be a challenge with almost one third of children not started on the preventive therapy.

The six month longitudinal study that assessed the impact of helminth and tuberculosis infection on child growth, micronutrients status and cognitive function showed one third of children to have vitamin A deficiency and almost one fourth had ferritin deficiency. The co-existing helminth infection was shown to impair serum ferritin recovery after six month of follow-up. The four components of cognitive function showed varying improvement with different variables, higher improvement was observed with helminth infection, anemia, vitamin A and deworming.

Discussion

Table 12. The contribution of this PhD thesis to the nexus of the Swiss TPH built around the triangle of innovation, validation and application.

Chapter	Title	Innovation	Findings/Validation	Application/Policy
2	*Schistosoma,* other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania	We have used a POC-CCA in settings where *S. mansoni* transmission has not been reported previously.	About one quarter of children were found to have *S. mansoni* infection as determined by POC-CCA	Use of antigen tests such as POC-CCA or other assays should be considered to establish the undocumented burden of the Schistosomiasis towards elimination. Further validation of test in settings with low transmission of *S. mansoni* in young age groups to be considered
3	Immunologic-based diagnosis of latent tuberculosis among children under five years of age exposed and unexposed to tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening		A similar proportion of LTBI among children with and without documented TB exposure suggesting non-household transmission of TB	Use of IGRAs for LTBI diagnosis among TB unexposed under-fives in high TB transmission settings
4	Impact of infections on growth development, micronutrient status and cognitive function in pre-school aged children in Tanzania		Early intervention leads to micronutrients and cognitive function recovery among under-fives	Diagnosis and management of risk factors for impaired growth and cognitive function should be considered in children growth monitoring to prevent suboptimal growth, development and performance

IGRA: Interferon gamma release assay; LTBI: Latent tuberculosis infection; POC-CCA: Point of care circulating cathodic antigen: TB: Tuberculosis

5.2. High prevalence of *S. mansoni* and other helminth infections in urban setting in Dar es Salaam

To our knowledge, we are reporting results of a first study to use POC-CCA to diagnose *Schistosoma* infection among under-fives in the coastal urban region of Dar es Salaam in Tanzania. The POC-CCA test was primarily developed to detect CCA of *S. mansoni*. The reported prevalence of *S. mansoni* in Dar es Salaam is high. Cross-reactivity of POC-CCA among pregnant women has been reported (Greter et al., 2016). A recent systematic review has reported POC-CCA cross-reactivity between *S. mansoni* and *S. haematobium* in other populations (Ochodo et al., 2015), but possibility of *S. mansoni* infection in Dar es Salaam cannot be ruled out. In our results, we did not find *S. mansoni* eggs by the Kato-Katz method among our study population. The POC-CCA test has higher sensitivity than the conventional microscopy using the Kato-Katz method in diagnosing *S. mansoni* (Coulibaly et al., 2011). In studies that used both POC-CCA and Kato-Katz, the POC-CCA revealed a higher prevalence compared to Kato-Katz (Coulibaly et al., 2013). POC-CCA test has also been widely used elsewhere and even in Tanzania (Coulibaly et al., 2011, Colley et al., 2013, Coulibaly et al., 2013, Ruganuza et al., 2015). In a study that evaluated the POC-CCA in five countries, the test was shown to have a sensitivity of up to 100% and showed no cross-reactivity with concurrent *S. haematobium* infection (Colley et al., 2013). Whether cross-reactivity between *S. mansoni* and *S. haematobium* or with something else other than *S. haematobium* is the case in Dar es Salaam, this remains to be further in studied.

The high prevalence of 31% of *S. mansoni* as determined by POC-CCA we found could partly be explained by the presence of *S. mansoni* infection in similar settings as reported by Mhimbira et al. (Mhimbira et al., 2017). We used similar suite of standardized, quality-controlled diagnostic methods with an enhanced accuracy of species-specific helminth detection for our study. It should also be noted that urban schistosomiasis due to *S. mansoni* is not a new finding; it has been previously reported as diagnosed by Kato-Katz method among other populations elsewhere including Brazil (Cabello et al., 2016), Côte d'Ivoire (Matthys et al., 2007) even in Tanzania (Olsen et al., 2015). Despite reports of cross-reactivity of *S. mansoni* and *S. haematobium* with a POC-CCA test in other settings (Ochodo et al., 2015, Greter et al., 2016), we

are reporting a very low prevalence of *S. haematobium* infection (1%) as determined by urine filtration, only one child is reported to have been co-infected with both *S. mansoni* (POC-CCA positive) and *S. haematobium*. In a recent study in Dar es Salaam that reported a similar low prevalence of 1.2% of *S. haematobium* among school-aged children, no report of S. *mansoni* was found despite the use of Kato-Katz (Mwakitalu et al., 2014).

Dar es Salaam is known to be a high *S. haematobium* prevalent setting, and no *Biomphalaria* snails were documented to inhabit the fresh water bodies of the city (Sarda et al., 1985, Mazigo et al., 2012). This is attributed to climatic conditions of the coastal region and the presence of sea water from the Indian Ocean which is not habitable for *Biomphalaria* snails. Given the limitation of use of POC-CCA test, but since the test detects *Schistosoma* antigens, possibly *Schistosoma* infection is high in this younger population. It will then be worthwhile to confirm our findings with other advanced and more sensitive tests such as circulating anodic antigen test or serology.

As presented in chapter 2, the prevalence of other helminths infection was 9%. This was lower than the recently reported prevalence in other under-resourced settings (Yirgalem G/Hiwot et al., 2014, Alemu et al., 2016). Even lower than what was reported 10 years ago in Dar es salaam where a study reported a prevalence of almost 50% of soil transmitted helminths in the other two districts in the region among the same population of children under the age of five years attending outpatient clinics (Kalison and Mwambete, 2006). Since 2004, the Ministry of Health in Tanzania and the United Nations Children Fund (UNICEF) has been providing bi-annual MDA across the country. During the MDA, mebendazole is distributed among children 6-59 months old. In parallel also oral vitamin A supplement is provided (Masanja et al., 2008). Therefore, the low prevalence of soil transmitted helminths infection in our settings can be explained by the MDA but also by improved socioeconomic status and better living conditions.

Contrary to other studies, (Davis et al., 2014, Ruganuza et al., 2015, Worrell et al., 2016, Alemu et al., 2016), we did not find any association between *S. mansoni* as well as other helminths and commonly reported risk factors such as age, hygiene, low socioeconomic status and contact water sources for

household chores. The lack of association between helminth and commonly reported risk factors may partly be explained by our sampling strategy. Our study was primarily powered to detect the prevalence of helminth infection among children under the age of five years rather than association with risk factors. The identified association of *Schistosoma* infection and septic tank toilets is hard to explain. First, because there was borderline significance when POC-CCA trace cases were considered negative and lacked statistical significance when POC-CCA trace results were included in the definition of *S. mansoni* infection. Schistosomiasis transmission requires human contact with contaminated natural open freshwater source where intermediate host snails inhabit. Contact of human and septic tanks does not explain schistosomiasis transmission. Potentially, having a septic tank toilet might be an indirect risk factor, considering that safe disposal of excreta in Dar es Salaam remains a challenge (Chaggu et al., 2002, Ministry of Health Community Development Gender Elderly and Children et al., 2016). Flooding of septic tanks in the rainy season might result in the contamination of nearby natural open freshwater sources with feces and potentially *S. mansoni* eggs, and subsequent infection of intermediate host snails and finally humans that come in contact with these waterbodies. Unfortunately, contact of preschool-aged children with natural open freshwater was not assessed in our study. The findings warrant further investigation to identify the presence of suitable intermediate host snails and hotspots of *Schistosoma* infection.

5.3. Prevalence of *M. tuberculosis* infection among exposed and unexposed under-fives

We show that, one fifth of children exposed to smear-positive infectious TB cases have LTBI. The reported prevalence is lower than what have been reported in South Africa where about a third of the children exposed to smear-positive infectious TB cases were diagnosed with LTBI based on a similar test, the QFT (Shah et al., 2011) and another study that reported a prevalence of almost 50% (Mandalakas et al., 2015). Children are among the high risk population of TB infection and have higher risk of progressing to TB disease if no preventive treatment is provided (Gessner et al., 1998, Marais et al., 2006). This had led to the WHO recommendation of TB contact screening which also includes under-fives (WHO, 2013). Various risk factors such as not being vaccinated with BGC, index being parent, smear-positivity of the index case,

and disease severity in the index case have been identified (Gessner et al., 1998, Hu et al., 2013). The low LTBI prevalence among children in our study population may be due to non-severe disease diagnosed in the index case and the fact that majority of children in our study were documented to have received the BCG vaccine. This could also be due to limited contact our study participants had with the index cases in their households as assessed by the score from South Africa (Mandalakas et al., 2012). Early diagnosis of pulmonary TB cases should be emphasized in the control of TB disease especially among younger children.

As part of TB screening strategy in our study, we also screened under-fives without documented TB exposure. We found out that, the prevalence of LTBI was similar to that of the other group of exposed under-fives. The WHO recommendation of contact screening implemented by many TB programs in high burden settings do not screen for TB infection in the absence of contact history within their households (Shah et al., 2011). Our findings of similar LTBI prevalence in the two groups of TB exposed and unexposed certainly suggest community TB transmission could be occurring in Dar es Salaam even in young children of less than five years who mostly stay indoors (Schaaf et al., 2003, Wood et al., 2010). Several studies show household TB transmission among younger children to be most common (Beyers et al., 1997, Wood et al., 2010, Fox et al., 2013, Jubulis et al., 2014). Non-household transmission of TB among young children can occur when a child visits multiple households other than their own; the risk is higher if the visited households have poor ventilation (Wood et al., 2010). Established community transmission of TB among younger children has consequences to TB infection screening strategy.

We were able to diagnose LTBI infection in unexposed children due to the use of an immunology test, the IGRA. IGRAs such as QFT and T-SPOT.TB are used in low-burden, high income settings to diagnose LTBI. Due to logistical constraints, programs in low resource settings do not include IGRAs in contact screening algorithms. Such tests are mainly used in research settings (Shah et al., 2011, Rose et al., 2012, Mandalakas et al., 2015). However, contact screening in such settings is mainly symptoms based. Similarly, Tanzanian government guidelines recommend symptomatic based TB screening of TB exposed children. TST is recommended whenever accessible (NTLP and MoHSW, 2012, NTLP and MoHSW, 2015).

As suggested in our results, we found children non-household transmission of TB does occur in Dar es Salaam, a region with high TB notification in the country (Wood et al., 2010). LTBI cases among such children can only be diagnosed by immunology tests such as QFT. This has cost implications to the program and TB control guidelines.

5.4. Uptake of isoniazid preventive therapy in under-fives exposed to tuberculosis

Our findings show that almost three quarters of children eligible for IPT based on history of exposure to infectious TB cases received the medication. TB infected children have high risk of progressing to TB disease if IPT is not given. Although not all TB exposed children were QFT positive, in many high TB burden countries TB screening is mainly symptom based and IPT 20mg/kg body weight is started in all under-fives irrespective of their immune status after ruling out an active disease. The 72% reported IPT uptake in our study is less than that set by the WHO of 90% (WHO, 2016a). In chapter 3, we show that uptake of IPT among LTBI children without documented TB exposure was almost zero. This may have been due to limited knowledge of the community as well as health care providers (Marais, 2017). While the WHO and its other stakeholders emphasize TB prevention in high risk populations, it is important that those with proven *M. tuberculosis* infection as diagnosed by recognized tests are identified, recorded in TB program registers and given IPT. Tanzanian NTLP together with its stakeholders give particular emphasis to under-five contact tracing. More recently, NTLP has improved childhood TB reporting and have a guideline on the management of TB in children (NTLP and MoHSW, 2012, NTLP and MoHSW, 2015). To further reduce TB incidence among this hard to diagnose, hard to treat population of children under-five, particular emphasis should be placed to ensure none infected miss the opportunity of being initiated on preventive medication.

5.5. Nutritional status in under-fives

Our results show that malnutrition remains to be a problem among under-fives even in one of the fastest growing commercial city of Dar es Salaam. According to the WHO classification of assessing severity of malnutrition by prevalence among under-fives (De Onis and Blossner, 2003), generally, the prevalence of

stunting was medium, underweight was high and that of wasting was very high; the prevalence of wasting was over 15%. All malnutrition indicators reported from our study are higher than those reported in the 2015-16 TDHS-MIS for Dar es Salaam region. According to the 2015-16 TDHS-MIS, the prevalence of stunting was 15%, wasting was 5% and underweight was 14%. Temeke, which is the focus of our study, is the poorest district in the Dar es Salaam (National Bureau of Statistics and Regional Commissioner's Office, 2014). Factors such as poor living conditions, infections, poor diet are said to affect children nutritional status, (Ayaya et al., 2004, Juma et al., 2016, Erismann et al., 2017).

Our findings show prevalence of vitamin A deficiency (VAD) of about one third of the studied under-fives. The reported prevalence is similar to that reported for Dar es Salaam in 2011 (National Bureau of Statistics et al., 2011). Vitamin A is an essential vitamin for growth, immune function and vision. Children in Tanzania receive vitamin A supplements twice a year as part of Tanzania Food and Nutrition Centre (TFNC) initiative against malnutrition and micronutrient deficiency (Masanja et al., 2008, Nyhus Dhillon et al., 2011). It has been seven years since the prevalence of 34% was reported, and despite multiple rounds of deworming and vitamin A supplementation (VAS), the prevalence has persistently remained the same. This level of VAD is still of public health importance and problematic given the fact that interventions have been ongoing in the country for the past 20 years and have been consistently reported to have high coverage of 90% of VAS (National Bureau of Statistics et al., 2011). Only recently, the coverage of VAS was estimated at 65% (Nyhus Dhillon et al., 2013). However, the prevalence of serum ferritin deficiency was 17%, about half that of vitamin A.

5.6. Helminth infections and its associations with micronutrient deficiency

We show that, after six months of study follow up, there was generally a decrease of serum ferritin and sTfR levels while vitamin A increase was observed. As presented earlier, the prevalence of anemia among our study population was almost four times that of serum ferritin deficiency at enrolment. It should be noted that, helminths infection was shown to be associated with anemia, similar to other findings (Ahmed et al., 2012, Alelign et al., 2015, Sayasone et al., 2015). While none of the infections such as helminth, malaria

and LTBI showed significant association with change in ferritin levels after a six month of follow-up time, also anemia had no association with ferritin recovery. Iron deficiency anemia may also occur due to low intake of iron in the meals (Mwangi et al., 2017). Organisms such as hookworm, *T. trichiura*, and *Schistosoma* that contribute to blood loss and *A. lumbricoides* that may cause intestinal damage and thus reducing surface area for nutrients absorption can lead to anemia in individuals (Koukounari et al., 2006, Yirgalem G/Hiwot et al., 2014). The prevalence of anemia was almost four times that of ferritin deficiency, and higher than that in the recent Tanzania Demographic and Health Survey and Malaria Indicator Survey report of 2015-16 (Ministry of Health Community Development Gender Elderly and Children et al., 2016). This can be explained first by the fact that, not all observed anemia cases among our study population were iron deficient anemia, other children presented with megaloblastic anemia. Second, as shown in chapter 2 of the thesis, the prevalence of STH was low and the reported cases of *S. mansoni* did not have eggs after examining stool samples by Kato-Katz.

As discussed above, our results show very low prevalence of *A. lumbricoides* of about 1%. The helminth was shown to be associated with the high prevalence of Vitamin A deficiency (Suchdev et al., 2014). However, VAD could also be due to a low intake of vitamin A in the diet given the observation that majority of children in developing countries are at a higher risk of having micronutrient deficiency due to marginal dietary intake. This may explain the lack of association between helminth and vitamin A deficiency that was observed both at enrollment and follow-up despite biannual VAS. It may also be that parents do not take their children for VAS. Vitamin A has been shown in a review to be associated with morbidity and mortality reduction in children (Imdad et al., 2017).

5.7. Policy implications

Our findings can have the following implications to the country's under-fives health policy:

- Under-fives are not included in Schistosomiasis deworming. The program should also consider including children younger than five years of age in MDA because of reported high prevalence of *S. mansoni* infection as determined by POC-CCA is alarming. However, there is a need to validate the POC-CCA in young age group in settings with low *S. mansoni* transmission.
- NTD should also consider use of more sensitive and specific tests or other advanced assays than the conventional Kato-Katz in areas with low *Schistosoma* prevalence in the ongoing MDA among school-aged population.
- Towards zero TB deaths, this is the time to diagnose as many children with TB infection as possible and start them on IPT to reduce the incidence of active TB disease. NTLP should consider use of IGRAs for LTBI diagnosis among unexposed TB children whenever resources allow.
- MoHSW and NTLP should consider integrating routine pediatric TB screening with child health clinics to bring more awareness to the community and advocate for early screening among young children suspected to have TB. This will provide a child friendly environment and remove one of the barriers in accessing TB screening services. Child health clinics should also be providing IPT.
- Tanzania was able to reach the millennium development goal of reducing child mortality attributing disease preventive interventions and improved social economic status (Afnan-Holmes et al., 2015). Early infection screening for helminth and tuberculosis and intervention is crucial to allow optimal growth and development. MoHSW should design settings specific screening algorithm to enable early interventions following diagnosis of underlying factors.
- Vitamin A supplementation has been shown to reduce morbidity and mortality. The reported high prevalence of VAD requires TFNC to expand the coverage of VAS to reduce the reported deficiency.

5.8. Synthesis of the results

The world has set its focus to ensure healthy lives and promote the well-being of all ages (Simkiss, 2015). While this is done, particular emphasis should be placed on younger children who are at a critical stage of development. In our study, we systematically screened children for TB, HIV, malaria and helminth infection, as all of these infections significantly contribute to under-five morbidity and mortality. Infections are a leading cause of morbidity among under-five population. We also assessed nutritional status and micronutrient deficiency of all our study population. Though the majority of these children were healthy at the time of study enrollment, they were identified from community settings and invited to the RCH clinic for consenting, enrollment and screening. The reported helminth prevalence of one quarter, anemia of two thirds as well VAD of one third as shown in Figure 15 below, all warrant immediate attention. The reported prevalence of LTBI of one fifth with similar proportion among under-fives with and without documented exposure to infectious TB cases is a matter of concern. Malnutrition prevalence adds to the growing concern of under-five health and wellbeing.

As is the case with other under-resourced settings, malnutrition which is highly prevalent worsens infection outcome (Jaganath and Mupere, 2012). Infection such as those due to helminth negatively impact nutritional status (Ibrahim et al., 2017) and plays a role in cognitive development (Nokes et al., 1992, Sakti et al., 1999, Ezeamama et al., 2005). In these settings, under-fives diets have insufficient nutritional density. The vicious cycle brought by infections, malnutrition, poor health and growth consequently prevent children from reaching their optimal developmental and cognition function milestones (Figure 16). A study in the Philippines reported association of helminth infection with three of the four cognitive components examined. *A. lumbricoides* was associated with poor memory, *Schistosoma* with poor learning and *T. trichiura* with poor performance of verbal fluency test while no effect was observed on non-verbal intelligence test (Ezeamama et al., 2005). Another study in Indonesia documented association of hookworm infection with memory; children diagnosed with hookworm infection performed poorly compared to their peers who did not have hookworm infection (Sakti et al., 1999). A study in Jamaica also reported *T.*

trichiura to be associated with poor cognitive performance which was reversed by anthelminthic (Nokes et al., 1992).

The co-existence of infections; helminth and tuberculosis, malnutrition and the observed poor growth and cognitive function pose a great challenge and detrimental to the wellbeing of under-fives who are at critical stage of growth and development.

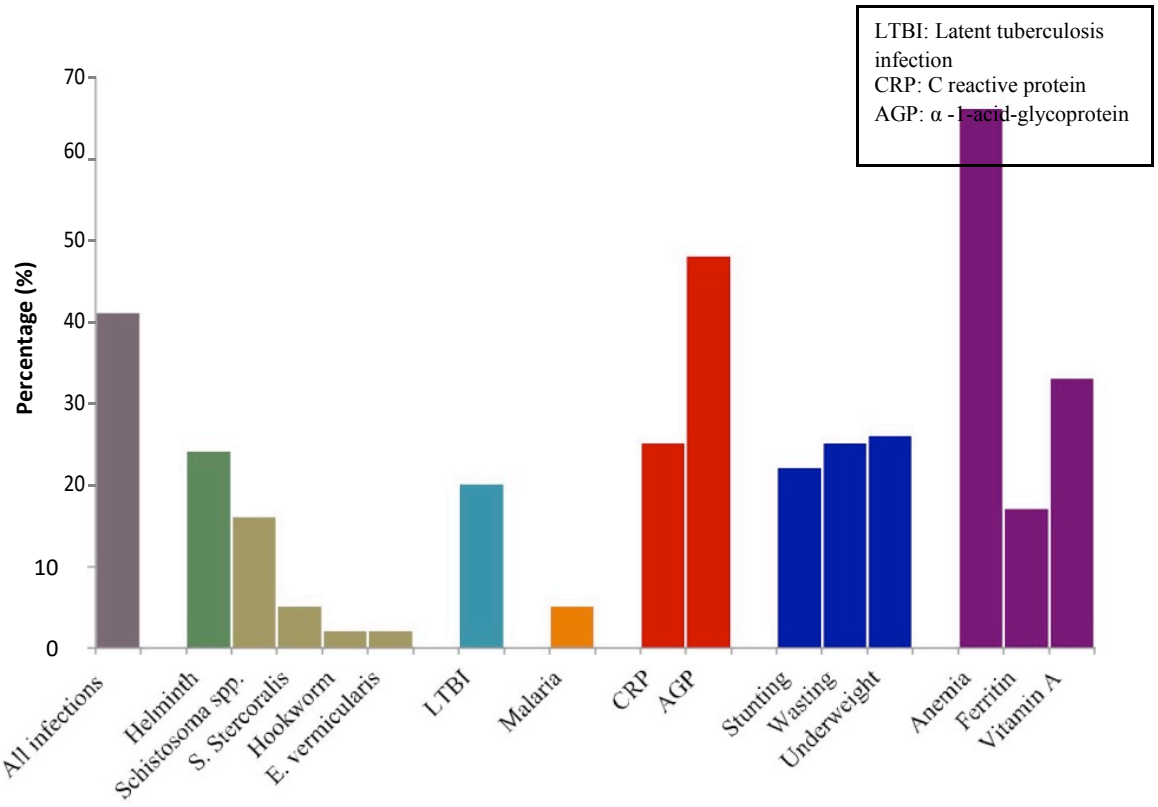


Figure 16. Frequency distribution of infections, inflammatory markers, nutritional indicators and micronutrient deficiencies among under-fives.

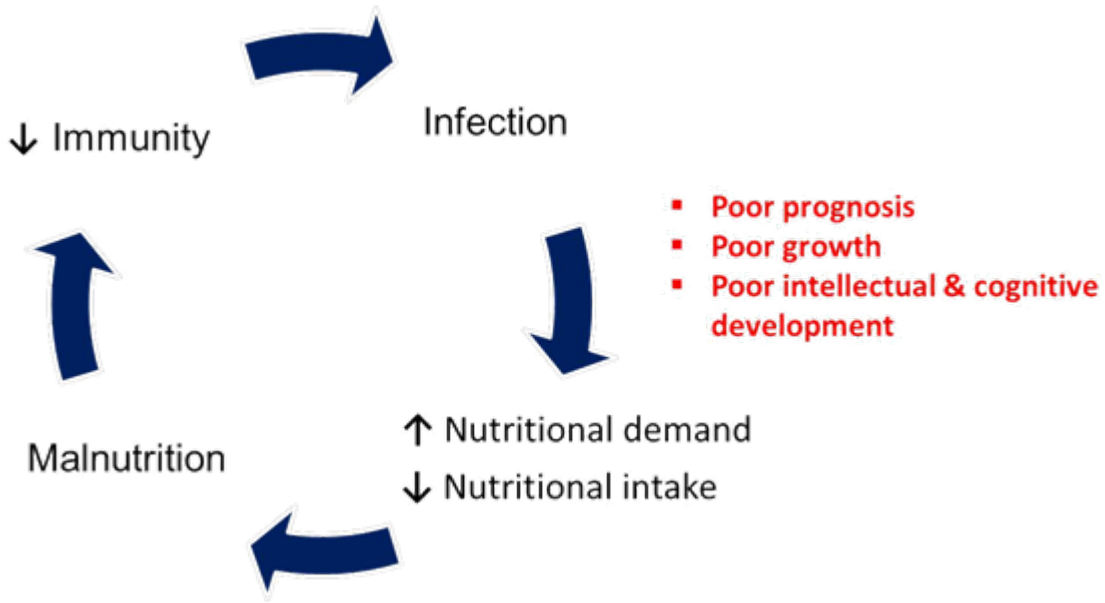


Figure 17. A schematic presentation of a vicious circle of infections, malnutrition and poor growth

5.9. Outlook and future perspective

Based on the discussion, limitations and policy implication discussed above, this PhD thesis has direct consequences to be considered:

- POC-CCA cross-reactivity of *S. mansoni* and *S. haematobium* and other conditions has recently been reported in other settings. This needs to be confirmed if it is the case for Dar es Salaam.
- To evaluate newly developed, more specific and sensitive, test assays to diagnose *Schistosoma* infection in low transmission settings such as Dar es Salaam.
- Assess other causes of anemia among under-fives to design appropriate intervention
- Design and test infections screening algorithm among under-fives to be used in RCH.

6. Conclusions and public health relevance of findings

Based on the results presented in the three papers of this PhD thesis and the discussion above, we would like to draw the following conclusions and suggest public health relevance of our findings.

6.1. Conclusions

The following are the main conclusions of this PhD thesis:

- The prevalence of helminth particularly *S. mansoni* among children younger than five years of age can be high in settings where *S. mansoni* has not been previously reported. This could possibly suggest a changing pattern of *Schistosoma* infection from predominantly *S. haematobium* to *S. mansoni* in Dar es Salaam.
- Soil transmitted helminth prevalence is declining. Sensitive diagnostic tools will become more important as the program moves towards STH elimination in the country.
- Children not documented to have been exposed to TB could be facing equal risk of TB infection from non-household TB transmission.
- IPT uptake among children exposed to an infectious TB case is below the WHO target of 90%. However, contact tracing of young children remains an important strategy to prevent active TB disease.
- The reported prevalence of Vitamin A deficiency has remained high despite several rounds of vitamin A supplementation in the country.
- The high prevalence of anemia does not match the prevalence of iron deficiency in our study population, indicating other possible causes such as diet and folic acid deficiency to have also contributed significantly to the problem.
- The improvement in cognitive function performance observed among children may be due to deworming, nutritional counselling and other medical treatment provided to under-fives. Early screening and intervention against helminth and tuberculosis infection among under-fives should be advocated and implemented for optimal growth development and cognitive function of the children.

- With the WHO's ambitious goal of reaching 100% coverage of preventive chemotherapy targeting major helminthiases, we call for urgent planning and implementation of specific interventions to prevent further morbidity, and to improve health, care, and wellbeing of these young children.

6.2. Public health relevance of findings

- Screening of *S. mansoni* with POC-CCA is feasible in health facility settings and should be considered for implementation.
- In settings with high TB notification rate like Temeke where TB exposure in the community is high, strategies to evaluate TB infection should not be limited to child contacts only.
- The NTLP should promote knowledge and emphasize IPT uptake and compliance among children eligible for treatment.
- TFNC should consider expanding VAS coverage to reduce the reported vitamin A deficiency.

7. Future research questions and recommendations

7.1. Identified research question to address key issues brought up in this PhD thesis.

7.1.1. Schistosoma mansoni transmission in Dar es Salaam

- Is there a change in transmission pattern of Schistosoma transmission in the city?
- Are we seeing cross-reactivity of POC-CCA test between *S. mansoni and S. haematobium* or with other conditions?
- Could the change be due to population migration from *S. mansoni* endemic regions to the city?
- Are there *Biompalaria* spp. snails inhabiting the open natural freshwater bodies of the city?
- Where does the contact with open natural freshwater bodies happen and transmission take place?
- What are the other risk factors for transmission *S. mansoni* schistosomiasis in the city?
- Is it time to include under-fives in the MDA against Schistosomiasis?

7.1.2. Non-household transmission of TB among under-fives

- Community TB transmission among under-fives is happening in Dar es Salaam. What implication will this have on IPT policy?
- How far should IPT coverage extend in the community?
- What are the cost implications of screening under-fives at RCH?
- What about IPT, is it time for all under-fives in settings with high TB notification?
- Should we wait for childhood incident TB cases or give IPT for non-household transmission?

7.1.3. Micronutrient deficiency and anemia

- Investigating the common cause of iron deficiency in Dar es Salaam
- Assessing the impact of folic acid supplementation among under-fives
- Assessing uptake of vitamin A supplementation among under-fives

Future research questions and recommendations

7.1.4. Cognitive function and growth development

- Designing simple tools to assess cognitive function among under-fives that can be used at child clinics
- Identifying the burden of reduced cognitive performance
- Developing algorithm for cognitive assessment of under-fives

8. References

Afnan-Holmes, H., Magoma, M., John, T., Levira, F., Msemo, G., Armstrong, C. E., Martínez-Álvarez, M., Kerber, K., Kihinga, C., Makuwani, A., Rusibamayila, N., Hussein, A., Lawn, J. E. & Tanzanian Countdown Country Case Study, G. 2015. Tanzania's countdown to 2015: an analysis of two decades of progress and gaps for reproductive, maternal, newborn, and child health, to inform priorities for post-2015. *The Lancet. Global Health,* 3**,** e396-409.

Ahmed, A., Al-Mekhlafi, H. M., Al-Adhroey, A. H., Ithoi, I., Abdulsalam, A. M. & Surin, J. 2012. The nutritional impacts of soil-transmitted helminths infections among Orang Asli schoolchildren in rural Malaysia. *Parasites & Vectors,* 5**,** 119.

Ahmed, F., Prendiville, N. & Narayan, A. 2016. Micronutrient deficiencies among children and women in Bangladesh: progress and challenges. *J Nutr Sci,* 5**,** e46.

Akman, M., Cebeci, D., Okur, V., Angin, H., Abali, O. & Akman, A. C. 2004. The effects of iron deficiency on infants' developmental test performance. *Acta Paediatr,* 93**,** 1391-6.

Al-Mekhlafi, M. S. H., Surin, J., Atiya, A. S., Ariffin, W. A., Mahdy, A. K. M. & Abdullah, H. C. 2008. Current prevalence and predictors of protein-energy malnutrition among schoolchildren in rural Peninsular Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health,* 39**,** 922-931.

Albonico, M., Allen, H., Chitsulo, L., Engels, D., Gabrielli, A. F. & Savioli, L. 2008. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis,* 2**,** e126.

Alelign, T., Degarege, A. & Erko, B. 2015. Soil-Transmitted Helminth Infections and Associated Risk Factors among Schoolchildren in Durbete Town, Northwestern Ethiopia. *Journal of Parasitology Research,* 2015.

Alemu, A., Tegegne, Y., Damte, D. & Melku, M. 2016. *Schistosoma mansoni* and soil-transmitted helminths among preschool-aged children in Chuahit, Dembia district, Northwest Ethiopia: prevalence, intensity of infection and associated risk factors. *BMC Public Health,* 16**,** 422.

Ali, S. S. 2013. A brief review of risk-factors for growth and developmental delay among preschool children in developing countries. *Adv Biomed Res,* 2**,** 91.

Anderson, R. M. & May, R. M. 1991. Infectious diseases of humans: dynamics and control. *Oxford***,** 768 p.

Anderson, S. T., Kaforou, M., Brent, A. J., Wright, V. J., Banwell, C. M., Chagaluka, G., Crampin, A. C., Dockrell, H. M., French, N., Hamilton, M. S., Hibberd, M. L., Kern, F., Langford, P. R., Ling, L., Mlotha, R., Ottenhoff, T. H. M., Pienaar, S., Pillay, V., Scott, J. a. G., Twahir, H., Wilkinson, R. J., Coin, L. J., Heyderman, R. S., Levin, M., Eley, B., Consortium, I. & Group, K. T. S. 2014. Diagnosis of childhood tuberculosis and host RNA expression in Africa. *The New England Journal of Medicine,* 370**,** 1712-1723.

Andrews, J. R., Bogoch, Ii & Utzinger, J. 2017. The benefits of mass deworming on health outcomes: new evidence synthesis, the debate persists. *Lancet Glob Health,* 5**,** e4-e5.

Arinola, G. O., Morenikeji, O. A., Akinwande, K. S., Alade, A. O., Olateru-Olagbegi, O., Alabi, P. E. & Rahamon, S. K. 2015. Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti-helminthic Treatment. *Ann Glob Health,* 81**,** 705-10.

Assey, V. D., Peterson, S., Kimboka, S., Ngemera, D., Mgoba, C., Ruhiye, D. M., Ndossi, G. D., Greiner, T. & Tylleskar, T. 2009. Tanzania national survey on iodine deficiency: impact after twelve years of salt iodation. *BMC Public Health,* 9**,** 319.

Awasthi, S., Peto, R., Read, S., Richards, S. M., Pande, V. & Bundy, D. 2013. Population deworming every 6 months with albendazole in 1 million pre-school children in North India: DEVTA, a cluster-randomized trial. *Lancet,* 381**,** 1478-86.

References

Ayaya, S. O., Esamai, F. O., Rotich, J. & Olwambula, A. R. 2004. Socio-economic factors predisposing under five-year-old children to severe protein energy malnutrition at the Moi Teaching and Referral Hospital, Eldoret, Kenya. *East Afr Med J,* 81**,** 415-21.

Babu, S. & Nutman, T. B. 2016. Helminth-Tuberculosis Co-infection: An Immunologic Perspective. *Trends Immunol,* 37**,** 597-607.

Bartram, J. & Cairncross, S. 2010. Hygiene, sanitation, and water: forgotten foundations of health. *PLoS Med,* 7**,** e1000367.

Becker, S. L., Yap, P., Horie, N. S., Alirol, E., Barbe, B., Bhatta, N. K., Bhattarai, N. R., Bottieau, E., Chatigre, J. K., Coulibaly, J. T., Fofana, H. K., Jacobs, J., Karki, P., Khanal, B., Knopp, S., Koirala, K., Mahendradhata, Y., Mertens, P., Meyanti, F., Murhandarwati, E. H., N'goran, E. K., Peeling, R. W., Pradhan, B., Ravinetto, R., Rijal, S., Sacko, M., Saye, R., Schneeberger, P. H., Schurmans, C., Silue, K. D., Steinmann, P., Van Loen, H., Verdonck, K., Van Lieshout, L., Von Muller, L., Yao, J. A., Boelaert, M., Chappuis, F., Polman, K. & Utzinger, J. 2016. Experiences and Lessons from a Multicountry NIDIAG study on persistent digestive disorders in the tropics. *PLoS Negl Trop Dis,* 10**,** e0004818.

Berhe, N., Myrvang, B. & Gundersen, S. G. 2009. Gastro-intestinal symptoms associated with intense *Schistosoma mansoni* infection affect class-attentiveness of schoolchildren in Ethiopia. *Acta Trop,* 110**,** 52-6.

Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D. & Hotez, P. J. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet,* 367**,** 1521-32.

Beyers, N., Gie, R. P., Schaaf, H. S., Van Zyl, S., Talent, J. M., Nel, E. D. & Donald, P. R. 1997. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. *Int J Tuberc Lung Dis,* 1**,** 38-43.

Bianchi, L., Galli, L., Moriondo, M., Veneruso, G., Becciolini, L., Azzari, C., Chiappini, E. & De Martino, M. 2009. Interferon-gamma release assay improves the diagnosis of tuberculosis in children. *Pediatr Infect Dis J,* 28**,** 510-4.

Booth, M., Vounatsou, P., N'goran E, K., Tanner, M. & Utzinger, J. 2003. The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing Schistosoma mansoni and hookworm co-infections in rural Cote d'Ivoire. *Parasitology,* 127**,** 525-31.

Borkow, G. & Bentwich, Z. 2000. Eradication of helminthic infections may be essential for successful vaccination against HIV and tuberculosis. *Bull World Health Organ,* 78**,** 1368-9.

Brooker, S. & Clements, A. C. 2009. Spatial heterogeneity of parasite co-infection: Determinants and geostatistical prediction at regional scales. *Int J Parasitol,* 39**,** 591-7.

Bundy, D., Sher, A. & Michael, E. 2000. Good worms or bad worms: do worm infections affect the epidemiological patterns of other diseases? *Parasitol Today,* 16**,** 273-4.

Cabello, R. K., Beck, L. C., Massara, C. L., Murta, F. L., Guimaraes, R. J., Pieri, O. S., Schall, V. T. & Favre, T. C. 2016. *Schistosoma mansoni* infection and related knowledge among schoolchildren in an endemic area of Minas Gerais, Brazil, prior to educational actions. *Acta Trop,* 164**,** 208-215.

Casmo, V., Augusto, G., Nala, R., Sabonete, A. & Carvalho-Costa, F. A. 2014. The effect of hookworm infection and urinary schistosomiasis on blood hemoglobin concentration of schoolchildren living in northern Mozambique. *Revista Do Instituto De Medicina Tropical De São Paulo,* 56**,** 219-224.

CDC 2010. Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection --- United States, 2010.

Chabala, C., Chongwe, G., Jumbe-Marsden, E. & Somwe, S. W. 2017. Missed opportunities for screening child contacts of smear-positive tuberculosis in Zambia, a high-prevalence setting. *Int J Tuberc Lung Dis,* 21**,** 53-59.

Chaggu, E., Mashauri, D., Van Buuren, J., Sanders, W. & Lettinga, G. 2002. Excreta disposal in Dar-es-Salaam. *Environ Manage,* 30**,** 609-20.

Chegou, N. N., Black, G. F., Loxton, A. G., Stanley, K., Essone, P. N., Klein, M. R., Parida, S. K., Kaufmann, S. H. E., Doherty, T. M., Friggen, A. H., Franken, K. L., Ottenhoff, T. H. & Walzl, G.

References

2012. Potential of novel Mycobacterium tuberculosis infection phase-dependent antigens in the diagnosis of TB disease in a high burden setting. *BMC Infect Dis,* 12**,** 10.

Clarke, N. E., Clements, A. C., Doi, S. A., Wang, D., Campbell, S. J., Gray, D. & Nery, S. V. 2017. Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis. *Lancet,* 389**,** 287-297.

Colley, D. G., Binder, S., Campbell, C., King, C. H., Tchuem Tchuente, L. A., N'goran, E. K., Erko, B., Karanja, D. M., Kabatereine, N. B., Van Lieshout, L. & Rathbun, S. 2013. A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. *Am J Trop Med Hyg,* 88**,** 426-32.

Coulibaly, J. T., Knopp, S., N'guessan, N. A., Silué, K. D., Fürst, T., Lohourignon, L. K., Brou, J. K., N'gbesso, Y. K., Vounatsou, P., N'goran, E. K. & Utzinger, J. 2011. Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire. *PLoS Negl Trop Dis,* 5**,** e1384.

Coulibaly, J. T., N'gbesso, Y. K., Knopp, S., N'guessan, N. A., Silue, K. D., Van Dam, G. J., N'goran, E. K. & Utzinger, J. 2013. Accuracy of urine circulating cathodic antigen test for the diagnosis of Schistosoma mansoni in preschool-aged children before and after treatment. *PLoS Negl Trop Dis,* 7**,** e2109.

Craig, J. M. & Scott, A. L. 2014. Helminths in the lungs. *Parasite Immunol,* 36**,** 463-474. Crompton, D. W. 1984. Influence of parasitic infection on food intake. *Federation Proceedings,* 43**,** 239-245.

Cuevas, L. E., Browning, R., Bossuyt, P., Casenghi, M., Cotton, M. F., Cruz, A. T., Dodd, L. E., Drobniewski, F., Gale, M., Graham, S. M., Grzemska, M., Heinrich, N., Hesseling, A. C., Huebner, R., Jean-Philippe, P., Kabra, S. K., Kampmann, B., Lewinsohn, D., Li, M., Lienhardt, C., Mandalakas, A. M., Marais, B. J., Menzies, H. J., Montepiedra, G., Mwansambo, C., Oberhelman, R., Palumbo, P., Russek-Cohen, E., Shapiro, D. E., Smith, B., Soto-Castellares, G., Starke, J. R., Swaminathan, S., Wingfield, C. & Worrell, C. 2012. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. *J Infect Dis,* 205 Suppl 2**,** S209-15.

Culha, G. & Sangün, M. K. 2007. Serum levels of zinc, copper, iron, cobalt, magnesium, and selenium elements in children diagnosed with Giardia intestinalis and Enterobiosis vermicularis in Hatay, Turkey. *Biological Trace Element Research,* 118**,** 21-26.

Cunha, A. J., Leite, A. J. & Almeida, I. S. 2015. The pediatrician's role in the first thousand days of the child: the pursuit of healthy nutrition and development. *J Pediatr (Rio J),* 91**,** S44-51.

Davis, S. M., Worrell, C. M., Wiegand, R. E., Odero, K. O., Suchdev, P. S., Ruth, L. J., Lopez, G., Cosmas, L., Neatherlin, J., Njenga, S. M., Montgomery, J. M. & Fox, L. M. 2014. Soil-Transmitted Helminths in Pre-School-Aged and School-Aged Children in an Urban Slum: A Cross-Sectional Study of Prevalence, Distribution, and Associated Exposures. *Am J Trop Med Hyg*.

De Onis, M. & Blossner, M. 2003. The World Health Organization Global Database on Child Growth and Malnutrition: methodology and applications. *Int J Epidemiol,* 32**,** 518-26.

Desalu, O. O., Adeoti, A. O., Fadeyi, A., Salami, A. K., Fawibe, A. E. & Oyedepo, O. O. 2013. Awareness of the Warning Signs, Risk Factors, and Treatment for Tuberculosis among Urban Nigerians. *Tuberc Res Treat,* 2013**,** 369717.

Diel, R., Loddenkemper, R., Niemann, S., Meywald-Walter, K. & Nienhaus, A. 2011. Negative and positive predictive value of a whole-blood interferon-gamma release assay for developing active tuberculosis: an update. *Am J Respir Crit Care Med,* 183**,** 88-95.

Diese, M., Shrestha, L., Pradhan, B., Singh, D., Raaijmakers, H., Kisesa, A., Chamla, D. & Ntambue, M. A. 2016. Bottlenecks and opportunities for delivering integrated pediatric HIV services in Nepal. *Curr Opin HIV AIDS,* 11 Suppl 1**,** S21-9.

Dinardo, A. R., Mace, E. M., Lesteberg, K., Cirillo, J. D., Mandalakas, A. M., Graviss, E. A., Orange, J. S. & Makedonas, G. 2016. Schistosome soluble egg antigen decreases *Mycobacterium tuberculosis*-

References

specific CD4+ T-Cell effector function with concomitant arrest of macrophage phago-lysosome maturation. *J Infect Dis,* 214**,** 479-88.

Dodd, P. J., Yuen, C. M., Sismanidis, C., Seddon, J. A. & Jenkins, H. E. 2017. The global burden of tuberculosis mortality in children: a mathematical modelling study. *Lancet Glob Health,* 5**,** e898-e906.

Elias, D., Mengistu, G., Akuffo, H. & Britton, S. 2006. Are intestinal helminths risk factors for developing active tuberculosis? *Trop Med Int Health,* 11**,** 551-8.

Erismann, S., Knoblauch, A. M., Diagbouga, S., Odermatt, P., Gerold, J., Shrestha, A., Tarnagda, G., Savadogo, B., Schindler, C., Utzinger, J. & Cisse, G. 2017. Prevalence and risk factors of undernutrition among schoolchildren in the Plateau Central and Centre-Ouest regions of Burkina Faso. *Infect Dis Poverty,* 6**,** 17.

Ezeamama, A. E., Friedman, J. F., Acosta, L. P., Bellinger, D. C., Langdon, G. C., Manalo, D. L., Olveda, R. M., Kurtis, J. D. & Mcgarvey, S. T. 2005. Helminth Infection and Cognitive Impairment Among Filipino Children. *Am J Trop Med Hyg,* 72**,** 540-548.

Fox, G. J., Barry, S. E., Britton, W. J. & Marks, G. B. 2013. Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J,* 41**,** 140-56.

Gessner, B. D., Weiss, N. S. & Nolan, C. M. 1998. Risk factors for pediatric tuberculosis infection and disease after household exposure to adult index cases in Alaska. *J Pediatr,* 132**,** 509-13.

Gladstone, M., Lancaster, G. A., Umar, E., Nyirenda, M., Kayira, E., Van Den Broek, N. R. & Smyth, R. L. 2010. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. *PLoS Med,* 7**,** e1000273.

Glinz, D., Wegmuller, R., Ouattara, M., Diakite, V. G., Aaron, G. J., Hofer, L., Zimmermann, M. B., Adiossan, L. G., Utzinger, J., N'goran, E. K. & Hurrell, R. F. 2017. Iron Fortified Complementary Foods Containing a Mixture of Sodium Iron EDTA with Either Ferrous Fumarate or Ferric Pyrophosphate Reduce Iron Deficiency Anemia in 12- to 36-Month-Old Children in a Malaria Endemic Setting: A Secondary Analysis of a Cluster-Randomized Controlled Trial. *Nutrients,* 9.

Graham, S. M., Ahmed, T., Amanullah, F., Browning, R., Cardenas, V., Casenghi, M., Cuevas, L. E., Gale, M., Gie, R. P., Grzemska, M., Handelsman, E., Hatherill, M., Hesseling, A. C., Jean-Philippe, P., Kampmann, B., Kabra, S. K., Lienhardt, C., Lighter-Fisher, J., Madhi, S., Makhene, M., Marais, B. J., Mcneeley, D. F., Menzies, H., Mitchell, C., Modi, S., Mofenson, L., Musoke, P., Nachman, S., Powell, C., Rigaud, M., Rouzier, V., Starke, J. R., Swaminathan, S. & Wingfield, C. 2012. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis,* 205 Suppl 2**,** S199-208.

Grantham-Mcgregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L. & Strupp, B. 2007. Developmental potential in the first 5 years for children in developing countries. *Lancet,* 369**,** 60-70.

Greter, H., Krauth, S. J., Ngandolo, B. N., Alfaroukh, I. O., Zinsstag, J. & Utzinger, J. 2016. Validation of a Point-of-Care Circulating Cathodic Antigen Urine Cassette Test for *Schistosoma mansoni* Diagnosis in the Sahel, and Potential Cross-Reaction in Pregnancy. *Am J Trop Med Hyg,* 94**,** 361-4.

Grimes, D. A. & Schulz, K. F. 2005. Compared to what? Finding controls for case-control studies. *Lancet,* 365**,** 1429-1433.

Guerrant, R. L., Oria, R. B., Moore, S. R., Oria, M. O. & Lima, A. A. 2008. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev,* 66**,** 487-505.

Harika, R., Faber, M., Samuel, F., Mulugeta, A., Kimiywe, J. & Eilander, A. 2017. Are Low Intakes and Deficiencies in Iron, Vitamin A, Zinc, and Iodine of Public Health Concern in Ethiopian, Kenyan, Nigerian, and South African Children and Adolescents? *Food Nutr Bull,* 38**,** 405-427.

Haustein, T., Ridout, D. A., Hartley, J. C., Thaker, U., Shingadia, D., Klein, N. J., Novelli, V.

References

& Dixon, G. L. 2009. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J,* 28**,** 669-73.

Hesham Al-Mekhlafi, M., Surin, J., Atiya, A. S., Ariffin, W. A., Mohammed Mahdy, A. K. & Che Abdullah, H. 2008. Pattern and predictors of soil-transmitted helminth reinfection among aboriginal schoolchildren in rural Peninsular Malaysia. *Acta Tropica,* 107**,** 200-204.

Hesseling, A. C. & Mandalakas, A. M. 2011. Interferon-gamma release assays for childhood tuberculosis: what does the future hold? *Int J Tuberc Lung Dis,* 15**,** 1423-4.

Hill, P. C. & Ota, M. O. 2010. Tuberculosis case-contact research in endemic tropical settings: design, conduct, and relevance to other infectious diseases. *Lancet Infect Dis,* 10**,** 723-32.

Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J. & Jacobson, J. 2008. Helminth infections: the great neglected tropical diseases. *J Clin Invest,* 118**,** 1311-1321.

Hotez, P. J., Damania, A., Barua, A. & Stanaway, J. 2017. The first "London Declaration": The Commonwealth and its neglected tropical diseases. *PLoS Negl Trop Dis,* 11**,** e0005321.

Hotez, P. J., Molyneux, D. H., Fenwick, A., Kumaresan, J., Ehrlich Sachs, S., Sachs, J. D. & Savioli, L. 2007. Control of neglected tropical diseases. *N Engl J Med,* 357**,** 1018-1027.

Hu, Y., Zhao, Q., Wu, L., Wang, W., Yuan, Z. & Xu, B. 2013. Prevalence of latent tuberculosis infection and its risk factors in schoolchildren and adolescents in Shanghai, China. *Eur J Public Health,* 23**,** 1064-9.

Hurwitz, J. L., Jones, B. G., Penkert, R. R., Gansebom, S., Sun, Y., Tang, L., Bramley, A. M., Jain, S., Mccullers, J. A. & Arnold, S. R. 2017. Low Retinol-Binding Protein and Vitamin D Levels Are Associated with Severe Outcomes in Children Hospitalized with Lower Respiratory Tract Infection and Respiratory Syncytial Virus or Human Metapneumovirus Detection. *J Pediatr*.

Ibrahim, M. K., Zambruni, M., Melby, C. L. & Melby, P. C. 2017. Impact of Childhood Malnutrition on Host Defense and Infection. *Clin Microbiol Rev,* 30**,** 919-971.

Imdad, A., Mayo-Wilson, E., Herzer, K. & Bhutta, Z. A. 2017. Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. *Cochrane Database Syst Rev,* 3**,** Cd008524.

Jaganath, D. & Mupere, E. 2012. Childhood tuberculosis and malnutrition. *J Infect Dis,* 206**,** 1809-15.

Jaganath, D., Zalwango, S., Okware, B., Nsereko, M., Kisingo, H., Malone, L., Lancioni, C., Okwera, A., Joloba, M., Mayanja-Kizza, H., Boom, W. H., Stein, C., Mupere, E. & Tuberculosis Research, U. 2013. Contact investigation for active tuberculosis among child contacts in Uganda. *Clin Infect Dis,* 57**,** 1685-1692.

Jubulis, J., Kinikar, A., Ithape, M., Khandave, M., Dixit, S., Hotalkar, S., Kulkarni, V., Mave, V., Gupte, N., Kagal, A., Jain, S., Bharadwaj, R. & Gupta, A. 2014. Modifiable risk factors associated with tuberculosis disease in children in Pune, India. *Int J Tuberc Lung Dis,* 18**,** 198-204.

Jukes, M. C., Nokes, C. A., Alcock, K. J., Lambo, J. K., Kihamia, C., Ngorosho, N., Mbise, A., Lorri, W., Yona, E., Mwanri, L., Baddeley, A. D., Hall, A. & Bundy, D. A. 2002. Heavy schistosomiasis associated with poor short-term memory and slower reaction times in Tanzanian schoolchildren. *Trop Med Int Health,* 7**,** 104-17.

Juma, O. A., Enumah, Z. O., Wheatley, H., Rafiq, M. Y., Shekalaghe, S., Ali, A., Mgonia, S. & Abdulla, S. 2016. Prevalence and assessment of malnutrition among children attending the Reproductive and Child Health clinic at Bagamoyo District Hospital, Tanzania. *BMC Public Health,* 16**,** 1094.

Kalison, K. D. & Mwambete, N. 2006. Prevalance of intestinal helminths infections among underfives and knowledge on helminthiases among mothers of the underfives in Dar es Salaam, Tanzania. *East Afri J Public Health,* 3**,** 8-11.

Kancheya, N., Luhanga, D., Harris, J. B., Morse, J., Kapata, N., Bweupe, M., Henostroza, G. & Reid, S. E. 2014. Integrating active tuberculosis case finding in antenatal services in Zambia. *Int J Tuberc Lung Dis,* 18**,** 1466-72.

References

Katz, N., Chaves, A. & Pellegrino, J. 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo,* 14**,** 397-400.

Knopp, S. 2011. *Diagnosis, Epidemiology and Control of Soil-Transmitted Helminth Infections in Zanzibar, Tanzania.* doctoral, University of Basel.

Knopp, S., Salim, N., Schindler, T., Karagiannis Voules, D. A., Rothen, J., Lweno, O., Mohammed, A. S., Singo, R., Benninghoff, M., Nsojo, A. A., Genton, B. & Daubenberger, C. 2014. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and *Strongyloides stercoralis* infections in Tanzania. *Am J Trop Med Hyg,* 90**,** 535-45.

Koukounari, A., Fenwick, A., Whawell, S., Kabatereine, N. B., Kazibwe, F., Tukahebwa, E. M., Stothard, J. R., Donnelly, C. A. & Webster, J. P. 2006. Morbidity indicators of Schistosoma mansoni: relationship between infection and anemia in Ugandan schoolchildren before and after praziquantel and albendazole chemotherapy. *Am J Trop Med Hyg,* 75**,** 278-86.

Kung'u, J. K., Wright, V. J., Haji, H. J., Ramsan, M., Goodman, D., Tielsch, J. M., Bickle, Q. D., Raynes, J. G. & Stoltzfus, R. J. 2009. Adjusting for the acute phase response is essential to interpret iron status indicators among young Zanzibari children prone to chronic malaria and helminth infections. *J Nutr,* 139**,** 2124-31.

Lienhardt, C., Fielding, K., Sillah, J., Tunkara, A., Donkor, S., Manneh, K., Warndorff, D., Mcadam, K. P. & Bennett, S. 2003. Risk factors for tuberculosis infection in sub-Saharan Africa: a contact study in The Gambia. *Am J Respir Crit Care Med,* 168**,** 448-55.

Lobato, L., Miranda, A., Faria, I. M., Bethony, J. M. & Gazzinelli, M. F. 2012. Development of cognitive abilities of children infected with helminths through health education. *Rev. Soc. Bras. Med. Trop.,* 45**,** 514-519.

Lobato, M. N., Mohle-Boetani, J. C. & Royce, S. E. 2000. Missed opportunities for preventing tuberculosis among children younger than five years of age. *Pediatrics,* 106**,** E75.

London Declaration 2012. http://thecommonwealth.org/sites/default/files/history-items/documents/London%20Declaration%20of%201949.pdf,.

Lonnroth, K., Jaramillo, E., Williams, B. G., Dye, C. & Raviglione, M. 2009. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med,* 68**,** 2240-6.

Lule, S. A., Mawa, P. A., Nkurunungi, G., Nampijja, M., Kizito, D., Akello, F., Muhangi, L., Elliott, A. M. & Webb, E. L. 2015. Factors associated with tuberculosis infection, and with anti-mycobacterial immune responses, among five year olds BCG-immunised at birth in Entebbe, Uganda. *Vaccine,* 33**,** 796-804.

Lustigman, S., Prichard, R. K., Gazzinelli, A., Grant, W. N., Boatin, B. A., Mccarthy, J. S. & Basáñez, M.-G. 2012. A research agenda for helminth diseases of humans: the problem of helminthiases. *PLoS Negl Trop Dis,* 6**,** e1582.

Mandalakas, A. M., Kirchner, H. L., Lombard, C., Walzl, G., Grewal, H. M., Gie, R. P. & Hesseling, A. C. 2012. Well-quantified tuberculosis exposure is a reliable surrogate measure of tuberculosis infection. *Int J Tuberc Lung Dis,* 16**,** 1033-9.

Mandalakas, A. M., Kirchner, H. L., Walzl, G., Gie, R. P., Schaaf, H. S., Cotton, M. F., Grewal, H. M. & Hesseling, A. C. 2015. Optimizing the detection of recent tuberculosis infection in children in a high tuberculosis-HIV burden setting. *Am J Respir Crit Care Med,* 191**,** 820-30.

Marais, B. J. 2017. Improving access to tuberculosis preventive therapy and treatment for children. *Int J Infect Dis,* 56**,** 122-125.

Marais, B. J., Gie, R. P., Schaaf, H. S., Beyers, N., Donald, P. R. & Starke, J. R. 2006. Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med,* 173**,** 1078-90.

Marais, B. J., Gie, R. P., Schaaf, H. S., Hesseling, A. C., Obihara, C. C., Starke, J. J., Enarson, D. A., Donald, P. R. & Beyers, N. 2004. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis,* 8**,** 392-402.

Marais, B. J. & Graham, S. M. 2014. Childhood tuberculosis: A roadmap towards zero deaths. *J Paediatr Child Health.*

References

Markus, M. B. & Fincham, J. E. 2007. Helminthiasis, bystander diseases and vaccines: analysis of interaction. *Trends in Parasitology,* 23**,** 517-519.

Marquez, C., Chamie, G., Achan, J., Luetkemeyer, A. F., Kyohere, M., Okiring, J., Dorsey, G., Kamya, M. R., Charlebois, E. D. & Havlir, D. V. 2016. Tuberculosis Infection in Early Childhood and the Association with HIV-exposure in HIV-uninfected Children in Rural Uganda. *Pediatr Infect Dis J,* 35**,** 524-9.

Masanja, H., De Savigny, D., Smithson, P., Schellenberg, J., John, T., Mbuya, C., Upunda, G., Boerma, T., Victora, C., Smith, T. & Mshinda, H. 2008. Child survival gains in Tanzania: analysis of data from demographic and health surveys. *Lancet,* 371**,** 1276-1283.

Masoumi Asl, H. M. M., Alborzi, A. M., Pourabbas, B. P. & Kalani, M. M. 2015. QuantiFERON-TB Gold and Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis Infection in Children. *Iran J Med Sci,* 40**,** 411-7.

Matthys, B., Tschannen, A. B., Tian-Bi, N. T., Comoé, H., Diabaté, S., Traoré, M., Vounatsou, P., Raso, G., Gosoniu, L., Tanner, M., Cisse, G., N'goran, E. K. & Utzinger, J. 2007. Risk factors for *Schistosoma mansoni* and hookworm in urban farming communities in western Côte d'Ivoire. *Trop Med Int Health,* 12**,** 709-723.

Mazigo, H. D., Nuwaha, F., Kinung'hi, S. M., Morona, D., Pinot De Moira, A., Wilson, S., Heukelbach, J. & Dunne, D. W. 2012. Epidemiology and control of human schistosomiasis in Tanzania. *Parasit Vectors,* 5**,** 274.

Mccarty, T. R., Turkeltaub, J. A. & Hotez, P. J. 2014. Global progress towards eliminating gastrointestinal helminth infections. *Curr. Opin. Gastroenterol.,* 30**,** 18-24.

Mcdonald, C. M., Manji, K. P., Kupka, R., Bellinger, D. C., Spiegelman, D., Kisenge, R., Msamanga, G., Fawzi, W. W. & Duggan, C. P. 2013. Stunting and wasting are associated with poorer psychomotor and mental development in HIV-exposed Tanzanian infants. *J Nutr,* 143**,** 204-14.

Mcsorley, H. J. & Maizels, R. M. 2012. Helminth Infections and Host Immune Regulation. *Clin. Microbiol. Rev.,* 25**,** 585-608.

Mejia Torres, R. E., Franco Garcia, D. N., Fontecha Sandoval, G. A., Hernandez Santana, A., Singh, P., Mancero Bucheli, S. T., Saboya, M. & Paz, M. Y. 2014. Prevalence and intensity of soil-transmitted helminthiasis, prevalence of malaria and nutritional status of school going children in honduras. *PLoS Negl Trop Dis,* 8**,** e3248.

Mghanga, F. P., Genge, C. M., Yeyeye, L., Twalib, Z., Kibopile, W., Rutalemba, F. J. & Shengena, T. M. 2017. Magnitude, Severity, and Morphological Types of Anemia in Hospitalized Children Under the Age of Five in Southern Tanzania. *Cureus,* 9**,** e1499.

Mhimbira, F., Hella, J., Said, K., Kamwela, L., Sasamalo, M., Maroa, T., Chiryamkubi, M., Mhalu, G., Schindler, C., Reither, K., Knopp, S., Utzinger, J., Gagneux, S. & Fenner, L. 2017. Prevalence and clinical relevance of helminth co-infections among tuberculosis patients in urban Tanzania. *PLoS Negl Trop Dis,* 11**,** e0005342.

Ministry of Health Community Development Gender Elderly and Children, Ministry of Health, National Bureau of Statistics, Office of the Chief Government Statistician & Icf 2016. Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16.

Mishra, P. K., Palma, M., Bleich, D., Loke, P. & Gause, W. C. 2014. Systemic impact of intestinal helminth infections. *Mucosal Immunol,* 7**,** 753-762.

MoHSW 2013a. First National Tuberculosis Prevalence Survey In the United Republic of Tanzania - Final Report.

MoHSW 2013b. Standard Treatment Guidelines and Essential Medicines List (4th Edition). MoHSW 2017. Strategic Master Plan for the Neglected Tropical Diseases Control Program 2017-2022 Tanzania Mainland. *Ministry of Health and Social Welfare*.

Morrison, J., Pai, M. & Hopewell, P. C. 2008. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. *Lancet Infect Dis,* 8**,** 359-368.

References

Mwakitalu, M. E., Malecela, M. N., Mosha, F. W. & Simonsen, P. E. 2014. Urban schistosomiasis and soil transmitted helminthiases in young school children in Dar es Salaam and Tanga, Tanzania, after a decade of anthelminthic intervention. *Acta Trop,* 133**,** 35-41.

Mwambete, K. D., Tunzo, J. & Justin-Temu, M. 2013. Prevalence and management of helminthiasis among underfives living with HIV/AIDS at Amana Hospital, Tanzania. *J Int Assoc Provid AIDS Care,* 12**,** 122-7.

Mwangi, M. N., Phiri, K. S., Abkari, A., Gbane, M., Bourdet-Sicard, R., Braesco, V. A., Zimmermann, M. B. & Prentice, A. M. 2017. Iron for Africa-Report of an Expert Workshop. *Nutrients,* 9.

Nakaoka, H., Lawson, L., Squire, S. B., Coulter, B., Ravn, P., Brock, I., Hart, C. A. & Cuevas, L. E. 2006. Risk for tuberculosis among children. *Emerg Infect Dis,* 12**,** 1383-8.

National Bureau of Statistics & Office of Chief Goverment Statistician 2013. Tanzania Population and Housing Census 2012.

National Bureau of Statistics & Office of Chief Government Statistician 2015. Mortality and Health.

National Bureau of Statistics & Regional Commissioner's Office 2014. *Dar es Salaam Region Socio-economic Profile*

National Bureau of Statistics, Tanzania & Macro, I. 2011. Micronutrients: Results of the 2010 Tanzania Demographic and Health Survey.

Nga, T. T., Winichagoon, P., Dijkhuizen, M. A., Khan, N. C., Wasantwisut, E. & Wieringa, F. T. 2011. Decreased Parasite Load and Improved Cognitive Outcomes Caused by Deworming and Consumption of Multi-Micronutrient Fortified Biscuits in Rural Vietnamese Schoolchildren. *Am J Trop Med Hyg,* 85**,** 333-340.

Nguyen, T. H., Odermatt, P., Slesak, G. & Barennes, H. 2009. Risk of latent tuberculosis infection in children living in households with tuberculosis patients: a cross sectional survey in remote northern Lao People's Democratic Republic. *BMC Infect Dis,* 9**,** 96.

Nokes, C., Grantham-Mcgregor, S. M., Sawyer, A. W., Cooper, E. S. & Bundy, D. A. 1992. Parasitic helminth infection and cognitive function in school children. *Proc Biol Sci,* 247**,** 77-81.

NTLP & MoHSW 2012. National Guidelines for the Management of Tuberculosis in Children (1st edition). *National Tuberculosis and Leprosy Programme and Ministry of Health and Social Welfare*.

NTLP & MoHSW 2015. National Tuberculosis and Leprosy 2014 Annual Report. Dar es Salaam: Ministry of Health and Social Welfare.

Nyhus Dhillon, C., Subramaniam, H., Klemm, R., Rambeloson, Z. & Mulokozi, G. 2011. Vitamin A Supplementation & De-worning Post Event Coverage Survey-2010 Tanzania Mainland.

Nyhus Dhillon, C., Subramaniam, H., Mulokozi, G., Rambeloson, Z. & Klemm, R. 2013. Overestimation of vitamin A supplementation coverage from district tally sheets demonstrates importance of population-based surveys for program improvement: lessons from Tanzania. *PLoS One,* 8**,** e58629.

Ochodo, E. A., Gopalakrishna, G., Spek, B., Reitsma, J. B., Van Lieshout, L., Polman, K., Lamberton, P., Bossuyt, P. M. & Leeflang, M. M. 2015. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. *Cochrane Database Syst Rev***,** CD009579.

Olds, G. R. 2013. Deworming the world. *Trans. Am. Clin. Climatol. Assoc.,* 124**,** 265-274.

Oliveira, C. D., Melo, A. C., Oliveira, L. R., Froede, E. L. & Camargos, P. 2015. Control measures to trace </= 15-year-old contacts of index cases of active pulmonary tuberculosis. *J Bras Pneumol,* 41**,** 449-53.

Olsen, A., Kinung'hi, S. & Magnussen, P. 2015. *Schistosoma mansoni* infection along the coast of Lake Victoria in Mwanza region, Tanzania. *Am J Trop Med Hyg,* 92**,** 1240-4.

Pai, M., Behr, M. A., Dowdy, D., Dheda, K., Divangahi, M., Boehme, C. C., Ginsberg, A., Swaminathan, S., Spigelman, M., Getahun, H., Menzies, D. & Raviglione, M. 2016. Tuberculosis. *Nat Rev Dis Primers,* 2**,** 16076.

Pai, M., Dheda, K., Cunningham, J., Scano, F. & O'brien, R. 2007. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. *Lancet Infect Dis,* 7**,** 428-438.

References

Paynter, S., Ware, R. S., Lucero, M. G., Tallo, V., Nohynek, H., Weinstein, P., Williams, G., Sly, P. D. & Simoes, E. A. 2014. Malnutrition: a risk factor for severe respiratory syncytial virus infection and hospitalization. *Pediatr Infect Dis J,* 33**,** 267-71.

Perez-Porcuna, T. M., Ascaso, C., Malheiro, A., Abellana, R., Martins, M., Sardinha, J. F., Quinco, P., Antunes, I. A., Da Silva Garrido, M., Buhrer-Sekula, S. & Martinez-Espinosa, F. E. 2014. *Mycobacterium tuberculosis* infection in young children: analyzing the performance of the diagnostic tests. *PLoS One,* 9**,** e97992.

Pérez-Porcuna, T. M., Ascaso, C., Malheiro, A., Abellana, R., Martins, M., Sardinha, J. F. J., Quincó, P., Antunes, I. A., Da Silva Garrido, M., Bührer-Sékula, S. & Martinez-Espinosa, F. E. 2014. Mycobacterium tuberculosis Infection in Young Children: Analyzing the Performance of the Diagnostic Tests. *PLoS ONE,* 9**,** e97992.

Powell, D. A. 2009. Interferon gamma release assays in the evaluation of children with possible *Mycobacterium tuberculosis* infection: a view to caution. *Pediatr Infect Dis J,* 28**,** 676-7.

Pullan, R. L., Smith, J. L., Jasrasaria, R. & Brooker, S. J. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors,* 7**,** 37.

Rafi, W., Ribeiro-Rodrigues, R., Ellner, J. J. & Salgame, P. 2012. Coinfection-helminthes and tuberculosis. *Curr Opin HIV AIDS,* 7**,** 239-44.

Resende Co, T., Hirsch, C. S., Toossi, Z., Dietze, R. & Ribeiro-Rodrigues, R. 2007. Intestinal helminth co-infection has a negative impact on both anti-Mycobacterium tuberculosis immunity and clinical response to tuberculosis therapy. *Clin Exp Immunol,* 147**,** 45-52.

Righetti, A. A., Adiossan, L. G., Ouattara, M., Glinz, D., Hurrell, R. F., N'goran, E. K., Wegmüller, R. & Utzinger, J. 2013. Dynamics of Anemia in Relation to Parasitic Infections, Micronutrient Status, and Increasing Age in South-Central Côte d'Ivoire. *J Infect Dis.,* 207**,** 1604-1615.

Ritz, N. & Curtis, N. 2014. Novel concepts in the epidemiology, diagnosis and prevention of childhood tuberculosis. *Swiss Medical Weekly,* 144**,** w14000.

Rose, M. V., Kimaro, G., Nissen, T. N., Kroidl, I., Hoelscher, M., Bygbjerg, I. C., Mfinanga, S. G. & Ravn, P. 2012. QuantiFERON(R)-TB gold in-tube performance for diagnosing active tuberculosis in children and adults in a high burden setting. *PLoS One,* 7**,** e37851.

Ruganuza, D. M., Mazigo, H. D., Waihenya, R., Morona, D. & Mkoji, G. M. 2015. *Schistosoma mansoni* among pre-school children in Musozi village, Ukerewe Island, North-Western-Tanzania: prevalence and associated risk factors. *Parasit Vectors,* 8**,** 377.

Ruhago, G. M., Ngalesoni, F. N., Robberstad, B. & Norheim, O. F. 2015. Cost-effectiveness of live oral attenuated human rotavirus vaccine in Tanzania. *Cost Eff Resour Alloc,* 13**,** 7.

Sabi, I., Kabyemera, R., Mshana, S. E., Kidenya, B. R., Kasanga, G., Gerwing-Adima, L. E., Meremo, A., Clowes, P., Rachow, A. & Peck, R. N. 2016. Pulmonary TB bacteriologically confirmed by induced sputum among children at Bugando Medical Centre, Tanzania. *Int J Tuberc Lung Dis,* 20**,** 228-34.

Sachiyo Nagi, M. I. 2013. Relationship between *Mycobacterium tuberculosis* and hookworm infections among school children in Mbita, Kenya. *J Trop Dis*.

Said, K., Hella, J., Knopp, S., Nassoro, T., Shija, N., Aziz, F., Mhimbira, F., Schindler, C., Mwingira, U., Mandalakas, A. M., Manji, K., Tanner, M., Utzinger, J. & Fenner, L. 2017a. Schistosoma, other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania. *PLoS Negl Trop Dis,* 11**,** e0006017.

Said, K., Hella, J., Ruzegea, M., Solanki, R., Chiryamkubi, M., Mhimbira, F., Ritz, N., Schindler, C., Mandalakas, A. M., Manji, K. P., Tanner, M., Utzinger, J. & Fenner, L. 2017b. Immunologic-based Diagnosis of Latent Tuberculosis among Children Under Five Years of Age Exposed and Unexposed to Tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening. *Submitted*.

Sakti, H., Nokes, C., Subagio Hertanto, W., Hendratno, S., Hall, A., Bundy, D. a. P. & Satoto 1999. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Trop Med Int Health,* 4**,** 322-334.

References

Salgame, P., Yap, G. S. & Gause, W. C. 2013. Effect of helminth-induced immunity on infections with microbial pathogens. *Nat Immunol,* 14**,** 1118-26.

Salim, N., Schindler, T., Abdul, U., Rothen, J., Genton, B., Lweno, O., Mohammed, A. S., Masimba, J., Kwaba, D., Abdulla, S., Tanner, M., Daubenberger, C. & Knopp, S. 2014. Enterobiasis and strongyloidiasis and associated co-infections and morbidity markers in infants, preschool- and school-aged children from rural coastal Tanzania: a cross-sectional study. *BMC Infect Dis,* 14**,** 644.

Sarda, R. K., Simonsen, P. E. & Mahikwano, L. F. 1985. Urban transmission of urinary schistosomiasis in Dar es Salaam, Tanzania. *Acta Trop,* 42**,** 71-78.

Sayasone, S., Utzinger, J., Akkhavong, K. & Odermatt, P. 2015. Multiparasitism and intensity of helminth infections in relation to symptoms and nutritional status among children: A cross-sectional study in southern Lao People's Democratic Republic. *Acta Trop.,* 141**,** 322-331.

Schaaf, H. S., Michaelis, I. A., Richardson, M., Booysen, C. N., Gie, R. P., Warren, R., Van Helden, P. D. & Beyers, N. 2003. Adult-to-child transmission of tuberculosis: household or community contact? *Int J Tuberc Lung Dis,* 7**,** 426-31.

Schaible, U. E. & Kaufmann, S. H. 2007. Malnutrition and infection: complex mechanisms and global impacts. *PLoS Med,* 4**,** e115.

Scott, S. P., Chen-Edinboro, L. P., Caulfield, L. E. & Murray-Kolb, L. E. 2014. The impact of anemia on child mortality: an updated review. *Nutrients,* 6**,** 5915-32.

Seddon, J. A. & Shingadia, D. 2014. Epidemiology and disease burden of tuberculosis in children: a global perspective. *Infect Drug Resist,* 7**,** 153-65.

Shah, M., Kasambira, T. S., Adrian, P. V., Madhi, S. A., Martinson, N. A. & Dorman, S. E. 2011. Longitudinal analysis of QuantiFERON-TB Gold In-Tube in children with adult household tuberculosis contact in South Africa: a prospective cohort study. *PLoS One,* 6**,** e26787.

Simkiss, D. 2015. The Millennium Development Goals are Dead; Long Live the Sustainable Development Goals. *J Trop Pediatr,* 61**,** 235-7.

Singh, A. R., Kharate, A., Bhat, P., Kokane, A. M., Bali, S., Sahu, S., Verma, M., Nagar, M. & Kumar, A. M. 2017. Isoniazid Preventive Therapy among Children Living with Tuberculosis Patients: Is It Working? A Mixed-Method Study from Bhopal, India. *J Trop Pediatr*.

Smith, I. 2003. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. *Clin Microbiol Rev,* 16**,** 463-96.

Sommer, A. 2008. Vitamin a deficiency and clinical disease: an historical overview. *J. Nutr.,* 138**,** 1835-1839.

Steiner, A., Hella, J., Gruninger, S., Mhalu, G., Mhimbira, F., Cercamondi, C. I., Doulla, B., Maire, N. & Fenner, L. 2016. Managing research and surveillance projects in real-time with a novel open-source eManagement tool designed for under-resourced countries. *J Am Med Inform Assoc,* 23**,** 916-23.

Stephenson, L. S., Latham, M. C. & Ottesen, E. A. 2000. Malnutrition and parasitic helminth infections. *Parasitology,* 121 Suppl**,** S23-38.

Stephenson, L. S., Latham, M. C. & Ottesen, E. A. 2001. Malnutrition and parasitic helminth infections. *Parasitology,* 121**,** S23.

Strunz, E. C., Suchdev, P. S. & Addiss, D. G. 2016. Soil-Transmitted Helminthiasis and Vitamin A Deficiency: Two Problems, One Policy. *Trends Parasitol,* 32**,** 10-8.

Suchdev, P. S., Davis, S. M., Bartoces, M., Ruth, L. J., Worrell, C. M., Kanyi, H., Odero, K., Wiegand, R. E., Njenga, S. M., Montgomery, J. M. & Fox, L. M. 2014. Soil-transmitted helminth infection and nutritional status among urban slum children in Kenya. *Am J Trop Med Hyg,* 90**,** 299-305.

Taylor-Robinson, D. C., Maayan, N., Soares-Weiser, K., Donegan, S. & Garner, P. 2012. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. *Cochrane Database Syst Rev,* 11**,** CD000371.

Thomas, T. A., Mondal, D., Noor, Z., Liu, L., Alam, M., Haque, R., Banu, S., Sun, H. & Peterson, K. M. 2010. Malnutrition and helminth infection affect performance of an interferon gamma-release assay. *Pediatrics,* 126**,** e1522-9.

References

Thurnham, D. I., Mccabe, G. P., Northrop-Clewes, C. A. & Nestel, P. 2003. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. *Lancet,* 362**,** 2052-8.

Thurnham, D. I., Mccabe, L. D., Haldar, S., Wieringa, F. T., Northrop-Clewes, C. A. & Mccabe, G. P. 2010. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr,* 92**,** 546-55.

Tristao-Sa, R., Ribeiro-Rodrigues, R., Johnson, L. T., Pereira, F. E. & Dietze, R. 2002. Intestinal nematodes and pulmonary tuberculosis. *Rev Soc Bras Med Trop,* 35**,** 533-5.

Utzinger, J., Becker, S. L., Knopp, S., Blum, J., Neumayr, A. L., Keiser, J. & Hatz, C. F. 2012. Neglected tropical diseases: diagnosis, clinical management, treatment and control. *Swiss Med Wkly,* 142**,** w13727.

Vaivada, T., Gaffey, M. F. & Bhutta, Z. A. 2017. Promoting Early Child Development With Interventions in Health and Nutrition: A Systematic Review. *Pediatrics,* 140. Verhagen, L. M., Hermans, P. W., Warris, A., De Groot, R., Maes, M., Villalba, J. A., Del Nogal, B., Van Den Hof, S., Mughini Gras, L., Van Soolingen, D., Pinelli, E. & De Waard, J. H. 2012. Helminths and skewed cytokine profiles increase tuberculin skin test positivity in Warao Amerindians. *Tuberculosis (Edinb),* 92**,** 505-12.

Walker, S. P., Wachs, T. D., Gardner, J. M., Lozoff, B., Wasserman, G. A., Pollitt, E. & Carter, J. A. 2007. Child development: risk factors for adverse outcomes in developing countries. *Lancet,* 369**,** 145-57.

Walson, J. L., Stewart, B. T., Sangaré, L., Mbogo, L. W., Otieno, P. A., Piper, B. K. S., Richardson, B. A. & John-Stewart, G. 2010. Prevalence and correlates of helminth co-infection in Kenyan HIV-1 infected adults. *PLoS Negl Trop Dis,* 4**,** e644.

Wessells, K. R., Hess, S. Y., Ouedraogo, Z. P., Rouamba, N., Ouedraogo, J. B. & Brown, K. H. 2014. Asymptomatic malaria infection affects the interpretation of biomarkers of iron and vitamin A status, even after adjusting for systemic inflammation, but does not affect plasma zinc concentrations among young children in Burkina Faso. *J Nutr,* 144**,** 2050-8.

WHO 2006. The WHO Child Growth Standards *World Health Organization*.

WHO 2008. Worldwide Prevalence of Anaemia 1993–2005. WHO Global Database on Anaemia. *WHO*.

WHO 2009. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. *WHO*.

WHO 2011a. Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity *World Health Organization*.

WHO 2011b. Helminth Control in School-age Children: A Guide for Managers of Control Programmes. *WHO*.

WHO 2013. Roadmap for childhood tuberculosis: towards zero deaths. *WHO*.

WHO 2014. WHO guidance for national tuberculosis programmes on the management of tubeculosis in children. *WHO,* Second edition.

WHO 2016a. Global tuberculosis report 2016. *WHO*.

WHO 2016b. Pneumonia.

WHO 2017a. Diarrhoeal disease. *WHO*.

WHO 2017b. Malaria in children under five.

WHO 2017c. Soil-transmitted helminth infections. Fact sheet. *WHO*.

WHO & Carter Centre 2008. Intergrated control of the neglected tropical diseases: a neglected opportunity ripe for action. Paper jointly prepared by WHO and the Carter Center presented to the Global Health and the United Nations meeting, May 8, 2008. *World Health Organization*.

Wood, R., Johnstone-Robertson, S., Uys, P., Hargrove, J., Middelkoop, K., Lawn, S. D. & Bekker, L. G. 2010. Tuberculosis transmission to young children in a South African community: modeling household and community infection risks. *Clin Infect Dis,* 51**,** 401-8.

Worrell, C. M., Wiegand, R. E., Davis, S. M., Odero, K. O., Blackstock, A., Cuéllar, V. M., Njenga, S. M., Montgomery, J. M., Roy, S. L. & Fox, L. M. 2016. A Cross-sectional study of water, sanitation,

References

and hygiene-related risk factors for soil-transmitted helminth infection in urban school- and preschool-aged children in Kibera, Nairobi. *PLoS One,* 11**,** e0150744.

Yentur Doni, N., Yildiz Zeyrek, F., Simsek, Z., Gurses, G. & Sahin, I. 2015. Risk factors and relationship between intestinal parasites and the growth retardation and psychomotor development delays of children in Sanliurfa, Turkey. *Turkiye Parazitol Derg,* 39**,** 270-276.

Yimam, Y., Degarege, A. & Erko, B. 2016. Effect of anthelminthic treatment on helminth infection and related anaemia among school-age children in northwestern Ethiopia. *BMC Infect Dis,* 16**,** 613.

Yirgalem G/Hiwot, Degarege, A. & Erko, B. 2014. Prevalence of intestinal parasitic infections among children under five years of age with emphasis on *Schistosoma manson*i in Wonji Shoa Sugar Estate, Ethiopia. *PLoS One,* 9**,** e109793.

Zaman, M. M., Bhuiyan, M. R., Karim, M. N., Moniruzzaman, Rahman, M. M., Akanda, A. W. & Fernando, T. 2015. Clustering of non-communicable diseases risk factors in Bangladeshi adults: An analysis of STEPS survey 2013. *BMC Public Health,* 15**,** 659.

Zar, H. J., Hanslo, D., Apolles, P., Swingler, G. & Hussey, G. 2005. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. *Lancet,* 365**,** 130-4.

Zimmermann, M. B. 2011. The role of iodine in human growth and development. *Semin Cell Dev Biol,* 22**,** 645-52.

9. Appendices

Appendix 1: Standard Operating Procedure: Invitation of control children

SOP Title: TB/Helminth – FIELD WORK- Control child invitation

SOP code: BRTC _TBHelminth_002_V01

1.0 Introduction

TB/helminth is a cohort of children exposed and not exposed to sputum smear-positive adult TB cases in Temeke, Dar es Salaam. The study aims at determining the prevalence and intensity of intestinal helminth in children under-five exposed and not exposed to smear-positive TB cases. Furthermore, to assess TB and helminths co-infection association with clinical outcomes and TB exposure outcome among children under-five in the Temeke district, Dar es Salaam, Tanzania.

2.0 Objective

The objective of this SOP is to describe the randomization procedures a field worker will do to pick a control household during TB index case household visit when identifying children for possible enrollment into the TB/Helminth study.

3.0 Definition and Abbreviations

GPS**:** Geographical Positioning System

ODK: Open Data Kit

SOP: Standard Operation Procedure

TB: Tuberculosis

4.0 Scope

This SOP is applicable to all staff involved in field activities such as household visits and acquiring GPS coordinates. The SOP applied to field workers, study secretary and study clinician. The visit will be declared complete when all procedures purposed for that particular visit are done and checklist is completed.

Appendices

5.0 Responsibilities

The Principal Investigator will have overall responsibility for the procedures in this SOP. The study clinician will supervise the procedures on this SOP. The delegated staff responsible to perform the procedures described will be included in the site Delegation Log.

6.0 Study Procedures

All procedures and findings need to be entered into the Open Data Kit (ODK) to capture all the data generated by the study participants. A visit specific checklist and a sample collection checklist will guide through the procedures and completeness of the visit. Eligible participants who met all inclusion criteria and none of the exclusion criteria as per eligibility criteria will be enrolled into the TB-Dar study.

Visit	Day	Study Procedures	Location	Responsible Person
0	1	▪ After visiting the index case household, ▪ Using the sketch below visit the houses based on the pattern shown below ▪ Start by visiting the house on the right, number 1-9 ▪ Ask for the head of the household ▪ Enquire if there is any one >15 years old on **anti-TB medication** ▪ If there is, ask about TB treatment card and document Treatment number and thank the interviewee and go to the next house according to the sketch below ▪ If there is none proceed ▪ Enquire if there is a child 6-59 months old in the household and ▪ Talk to the parent or caretaker of the first under-five mentioned to you ▪ Follow the procedures of SOP BRTC_TBHelminth_001_V01 to proceed with invitation of parent/caretaker to Temeke District hospital for possible recruitment into the TB/Helminth study	Field	Field worker

Appendices

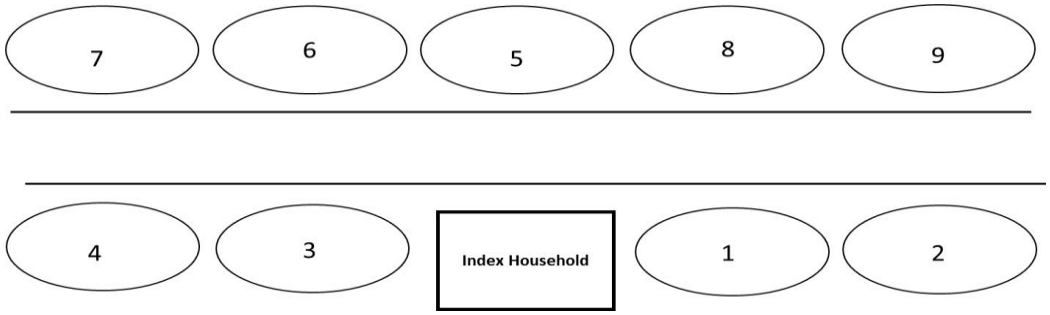


Figure 1: A sketch of the view of houses surrounding an index case household to be considered for randomization to recruit a child from a TB free household (Ref. 1).

Table 1. Table to register adults documented to have started antiTB during control participants recruitment

Date of household visit	Area visited	Number of adults on antiTB medication	NTLP TB treatment Number

Reference

1. Grimes DA, Schulz KF. Compared to what? Finding controls for case-control studies. The Lancet. 2005 Apr;365 (9468):1429–33.

Appendices

Appendix 2: Tuberculosis screening tool

Participant ID: 87__ __ __-31 **Participant initials: _____**

Symptomatic screening tool for possible TB disease in children

Screen all children during follow at 3, 6 & 12 months			
Symptom	**Present**	**Absent**	**If present**
Any current cough			**1. Treat, most likely alternative explanation** **2. Review after 1-2 week, if persistent – then full TB work-up**
Abnormal fatigue or reduced playfulness			
Documented weight loss or Failure to thrive in the past 3 months			
Current fever			**Review in 5-7 days, if persistent and malaria excluded – then full TB work-up**
Visible cervical mass (>2x2cm)			**If matted, non-painful nodes – then full TB work-up (including Fine Needle Aspiration Biopsy (FNAB))**
Gibbus (sharp angular spine deformity)			**Order spinal X-ray (PA and lateral views)**
Other signs suggestive of possible extra-pulmonary TB (EPTB) eg. distended abdomen with ascites, chronic osteo-articular disease or meningitis features _____			**Investigate as appropriate**

Full TB work-up for follow-up should include:

If PTB is suspected, chest X-ray (AP and lateral) and induced sputum

If EPTB is suspected include FNAB (for peripheral lymph node masses), Cerebral Spinal Fluid for TB Meningitis, or other direct sample of diseased tissues as possible/appropriate

10. Curriculum Vitae

Khadija S **IBRAHIM**

Contact Information
Bagamoyo Research and Training Centre (BRTC)
Ifakara Health Institute
P O Box 74
Bagamoyo
Coast
Tanzania
ORCID ID: 0000-0002-9236-8691

WORK EXPERIENCE

Visiting fellow (2017)	Laboratory of Human Nutrition, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland
PhD fellow (2014-17)	Swiss TPH/University of Basel, Basel, Switzerland
2009 to Present	Ifakara Health Institute, Dar es Salaam, Tanzania
Principal Investigator	Prevalence and clinical relevance of helminth and tuberculosis co-infection in children under five years of age in Tanzania
Investigator	Tuberculosis Cohort Study in the Dar es Salaam region (TB-DAR): a prospective collection of clinical data and biological specimens to study the epidemiology of tuberculosis, including molecular epidemiology and the evaluation of new diagnostics and biomarkers
Investigator	Epidemiology and Management of Tuberculosis in Tanzania (IHI/Swiss TPH)
Investigator	Evaluation of new and emerging diagnostics for childhood tuberculosis in high burden countries (TB CHILD)-EDCTP
Investigator	Phase II double-blind, randomized, placebo-controlled study to evaluate the safety and immunogenicity of H1/IC31®, an adjuvanted TB subunit vaccine, in HIV-infected adults with $cd4^+$ lymphocyte count greater than 350cells/mm3 - (SSI)
Principal Investigator	Estimating TB incidence among HIV-infected antiretroviral therapy naïve persons with early HIV disease in Bagamoyo district, Tanzania (EDCTP)
Investigator	Pharmacokinetics and pharmacodynamics of high versus standard dose rifampicin in patients with pulmonary tuberculosis in Tanzania (High RIF Study) - PANACEA
Investigator	A Phase II Open-Label Partially Randomized Trial to Evaluate the Efficacy, Safety and Tolerability of the combination of moxifloxacin plus PA-824 plus pyrazinamide after 8 weeks of treatment in Adult Patients with Newly Diagnosed Drug-Sensitive or Multi Drug-Resistant, Smear-Positive Pulmonary Tuberculosis. (TB ALLIANCE)
Research Officer	Phase III RTSs Malaria vaccine trial (GSK)

EDUCATION

Curriculum Vitae

PhD in Epidemiology	Swiss Tropical and Public Health Institute/ University of Basel October 2017, Basel, Switzerland
Masters in International Health	Swiss Tropical and Public Health Institute/ University of Basel August 2015, Basel, Switzerland
Medical Degree	Muhimbili University of Health and Allied Sciences December 2008, Dar es Salaam, Tanzania

PUBLICATIONS

1. **Said K**, Hella J, Knopp S, Nassoro T, Shija N, Aziz F, Mhimbira F, Schindler C, Mwingira U, Mandalakas AM, *et al.* Schistosoma, other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania. *PLoS Negl Trop Dis. 2017;11(11):e0006017.*
2. **Said K**, Hella J, Ruzegea M, Solanki R, Chiryamkubi M, Mhimbira F, Ritz N, Schindler C, Mandalakas AM, Manji KP, et al: Immunologic-Based Diagnosis of Latent Tuberculosis among Children Less Than 5 Years of Age Exposed and Unexposed to Tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening. *Pediatr Infect Dis J. 2018;* doi 10.1097/INF.0000000000002131
3. **Said K**, Hella J, Mhalu G, Chiryankubi M, Masika E, Maroa T, et al. Diagnostic delay and associated factors among patients with pulmonary tuberculosis in Dar es Salaam, Tanzania. Infect Dis Poverty. 2017;6(1):64.
4. Mhimbira F, Hella J, **Said K**, Kamwela L, Sasamalo M, Maroa T, et al. (2017) Prevalence and clinical relevance of helminth co-infections among tuberculosis patients in urban Tanzania. *PLoS Negl Trop Dis 11(2): e0005342. doi:10.1371/journal.pntd.0005342*
5. Mhimbira F, Hella J, Maroa T, Kisandu S, Chiryamkubi M, **Said K**, Fenner L. et al, Home-Based and Facility-Based Directly Observed Therapy of Tuberculosis Treatment under Programmatic Conditions in Urban Tanzania. *PLoS One, 2016. 11(8): p. e0161171.*
6. **Said K**, Verver S, Kalingonji A, Lwilla F, Mkopi A, Charalambous S, Reither K. Tuberculosis among HIV-infected population: incidence and risk factors in rural Tanzania. Afri Health Sci. 2017;17(1)
7. Leuenberger A, Nassoro T, **Said K**, Fenner L, Sikalengo G, Letang E, et al. Assessing stool quantities generated by three specific Kato-Katz thick smear templates employed in different settings. Infect Dis Poverty. 2016;5(1):58.
8. Reither K, Mfinanga E, **Said K**, Churchyard G, et al. Safety and immunogenicity of H1/IC31®, an adjuvanted TB subunit vaccine, in HIV-Infected adults with CD4+ lymphocyte counts greater than 350 cells/mm3: a phase II, multi-centre, double-blind, randomized, placebo-controlled trial. PLoS ONE 2014;9(12):e114602. doi:10.1371/journal.pone.0114602.
9. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, **Said K** et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis. 2014 October 29; 10.1016/S1473-3099(14)70884-9
10. Pohl C, Jugheli L, Haraka F, Mfinanga E, **Said K**, et al. (2013) Pulmonary Aspergilloma: A treatment challenge in sub-Saharan Africa. PLoS Negl Trop Dis 7(10): e2352. doi:10.1371/journal.pntd.0002352
11. **Said K**, Verver S, Churchyard G, Battegay M, Reither K et al**.** Improved services to enrollees into an HIV rural care and treatment center in Tanzania. The Pan Afr Med J. 2013;16:34. doi:10.11604/pamj.2013.16.34.1642

Curriculum Vitae

POSTER PRESENTATIONS

1. Said K, Mhimbira F, Hella J, Chiryamkubi M, Maroa T, Fenner L. Diagnostic delay and associated factors among patients with pulmonary tuberculosis in Dar es Salaam, Tanzania at the Union World Conference on Lung Health. Cape Town, South Africa 2-6 December 2015
2. Said K, Tanner M, Reither K. Tuberculosis Clinical Trial settings in Sub-Saharan Africa at the Novartis Institute of Tropical Diseases Tuberculosis Symposium. Yaoundé, Cameroun, 11– 15 October 2010

CONFERENCES AND SYMPOSIUM ATTENDED

1. The 46th Union World Conference on Lung Health. Cape Town, South Africa. 2-6 December 2015
2. TB and HIV: Breaking the vicious cycle by AIGHD, Amsterdam, The Netherlands. June 2012
3. Novartis Institute of Tropical Diseases Tuberculosis Symposium. Yaoundé, Cameroun, 11– 15 October 2010

COURSES ACCOMPLISHED DURING DOCTORAL TRAINING

Offered by	Course	Date	ECTS
University of Basel	Epidemiological Concepts	Sept 2014	3
	Health System	Sept 2014	2
	Concepts in Molecular Epidemiology	Sept 2014	2
	Biostatistics	Sept 2014	2
	Statistical modelling	Dec 2014	2
	Advances in Infection Biology, Epidemiology and Global Health	Sept 2014	1
	Data Analysis in Epidemiology	Jan 2015	2
	The Messenger is the message	March 2015	1
	Writing to be Published- Academic Writing, Convection and Style	March 2015	1
	Personal Profiler	March 2017	1
	Publishing Research Articles: Strategies and Steps	March 2017	1
	Job-Hunting	March 2017	1
	Getting Ready to Deal with People in Business	March 2017	1
	Writing Productivity: Tools and Techniques	April 2017	1
	Learning How to Lead and Building a Successful Work Environment	Sept 2017	1
	Becoming Your Own Time Manager	Oct 2017	-
	Total		**22**